

**CHARACTERIZATION AND IMPROVEMENT OF THE NUTRITIONAL
VALUE OF ETHANOL BY-PRODUCTS FOR SWINE**

A Thesis is Submitted to the College of
Graduate Studies and Research
In Partial Fulfilment of the Requirements
For the Degree of Master of Science
In the Department of Animal and Poultry Science
University of Saskatchewan
Saskatoon, Saskatchewan

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ABSTRACT

The nutritional value of distiller's dried grains with solubles (DDGS) has not been assessed in swine. The nutritional value of corn and wheat DDGS, and possibilities to improve the nutritional value of wheat DDGS were for swine were investigated in two studies.

In study 1, two experiments were conducted to determine digestibility and digestible contents of energy, amino acids (AA) and P in corn and wheat DDGS and wheat grain, together with N and P excretion and growth performance in grower-finisher pigs. In experiment 1, 12 barrows (64.6 ± 6.4 kg) were fitted with ileal T-cannulae and had restricted access (2.6 x maintenance) to a wheat control diet or one of three diets with 40% corn, wheat+corn (4:1) or wheat DDGS. For energy, apparent total tract digestibility was highest for wheat (85%; $P < 0.05$) and did not differ among DDGS (77 to 79%; $P > 0.10$). Total tract digestible energy (DE) was highest for corn DDGS (4292 kcal kg⁻¹ DM; $P < 0.05$) and tended to differ among wheat+corn and wheat DDGS and wheat (4038, 4019, and 3807, respectively; $P = 0.06$). For lysine, apparent ileal digestibility (AID) was highest for wheat (71%; $P < 0.05$) and did not differ among DDGS (59 to 63%; $P > 0.10$). The apparent ileal digestible lysine content was highest for corn DDGS (0.51% DM; $P < 0.05$), intermediate for wheat+corn and wheat DDGS (0.45 and 0.42), and lowest for wheat (0.37%). For P, total tract digestibility was lowest for wheat (15%; $P < 0.05$) and did not differ among DDGS samples (53 to 56%; $P > 0.10$). Total N excretion was highest for wheat+corn and wheat DDGS (55 and 58 g d⁻¹; $P < 0.05$), intermediate for corn DDGS (44) and lowest for wheat (36). Total P excretion did not differ among DDGS (11 g d⁻¹) and was lowest for wheat (8; $P < 0.05$). In

experiment 2, 100 pigs (52.0 ± 3.3 kg) were fed a wheat-pea control diet or one of three 25%-DDGS (corn, wheat+corn or wheat) diets (3.375 Mcal DE kg^{-1} ; 2.50 g AID lysine Mcal^{-1} DE) for 5 wk. Overall, average daily feed intake (ADFI) and daily gain (ADG) were higher for wheat than DDGS ($P < 0.05$) but feed efficiency did not differ ($P > 0.10$). In summary, the nutritional value of wheat DDGS for swine is higher than wheat and lower than corn DDGS and feeding DDGS reduced growth performance, partly via a reduced ADFI, indicating that anti-nutritional factors in DDGS require further investigation.

In study 2, the effect of xylanase supplementation of wheat DDGS on nutrient digestibility and nutrient excretion was evaluated in grower-finisher pigs. Wheat-based diets with or without 40% wheat DDGS were tested with or without supplementary xylanase ($4,000$ U kg^{-1} feed) as a 2×2 factorial arrangement in a repeated Latin square design using eight barrows (29.4 ± 2.0 kg) fitted with ileal T-cannulae. Following a 6-day acclimation, faeces and urine were collected for 3 d, and ileal digesta for 2 d. The apparent ileal energy digestibility and DE content were not affected either by ingredient or xylanase ($P > 0.05$). The total tract energy digestibility and DE content were affected by ingredient ($P > 0.05$), but not by xylanase ($P > 0.05$). The total-tract energy digestibility was higher for wheat, but DE content was higher for wheat DDGS. The AID of arginine, isoleucine, leucine, phenylalanine, threonine, tryptophan and total AA were higher ($P < 0.05$), and of cysteine, histidine and lysine were similar ($P > 0.05$), and SID of phenylalanine was higher ($P < 0.05$), and of the other AA was similar ($P > 0.10$) for wheat DDGS compared to wheat. Supplementary xylanase improved AID and SID of most of the indispensable AA in wheat ($P < 0.05$), but not in wheat DDGS ($P > 0.05$). The apparent and standardized ileal AA contents were affected by ingredients ($P <$

0.05), but not by xylanase ($P > 0.05$). Digestible AA contents were higher for wheat DDGS than for wheat. The digestibility and digestible content of P were affected by ingredient and xylanase ($P < 0.05$). The P digestibility and digestible P contents were higher for wheat DDGS compared to wheat. Neither ingredient nor supplementary xylanase affected DM intake ($P > 0.05$). The DM excretion on daily basis and as a percentage of intake were affected by ingredient ($P < 0.05$), but not by xylanase ($P > 0.05$). Ingredients affected all N and P variables ($P < 0.05$), except percentage retained for both nutrients ($P > 0.05$). None of N variables ($P > 0.05$), but P intake and, retention on daily basis and as a percentage of intake were affected by xylanase ($P < 0.05$). The DM excretion and N and P intake, excretion and daily retention were higher for wheat DDGS compared to wheat. Lack of beneficial response to supplementary xylanase might be due to inappropriate enzyme level or insufficient substrate level of wheat DDGS. In addition, unidentified factors associated with fermentation and drying processes might constrain the nutritional value of wheat DDGS. Further studies are required to determine the proper xylanase inclusion level and/or to identify the factors associated with reduced nutrient digestibility of wheat DDGS.

Key words: DDGS, pig, digestibility, energy, amino acid, xylanase

ACKNOWLEDGEMENTS

I would first and foremost like to express my sincere gratitude and appreciation to my supervisor Dr. Ruurd Zijlstra for his advice, guidance and encouragement during the entire Master's programme, which made the completion of this thesis possible. I would also like to thank Drs. Dave Christensen, Murray Drew and Fiona Buchanan for serving on my advisory committee, and Dr. Gordon Zello for serving as my external examiner.

I would like to acknowledge the Animal Nutrition Association of Canada for the generous scholarship I received during my studies. I would also like to recognize the financial support for this project from the Agriculture Development Fund of Saskatchewan Agriculture, Food and Rural Revitalization; and also strategic program funding from the pork producers of Saskatchewan, Manitoba and Alberta, together with Saskatchewan Agriculture and Food.

My most heartfelt thanks go to Mr. Brian Andries and barn staff, research scientists and office staff of the Prairie Swine Centre Inc. as well as the academic, laboratory and office staff of the Department of Animal and Poultry Science for their help in many senses that made possible to carry out and accomplish this endeavour.

I am greatly thankful to my fellow graduate students for their substantial cooperation, support and friendship. A special thanks also goes out to Ms. Mindy Spiehs of University of Minnesota for her invaluable assistance throughout the program.

I must also thank my sister, Manel and brother-in-law, Ransirimal for the support and encouragement received to initiate and finish this highly appreciated academic goal, and finally but most importantly, my dearest wife Dushmanthi and loving daughter Amaya for their love, support and above all their patience.

DEDICATION

I would like to dedicate this thesis to my parents, Eddie and Somalatha Widyaratne for having sown in me the principles of discipline, respect and admiration for life.

This work is also dedicated to all my past teachers, who encouraged my enthusiasm of learning from a very young age.

TABLE OF CONTENTS

PERMISSION TO USE STATEMENT	I
ABSTRACT	II
ACKNOWLEDGEMENTS	V
DEDICATION	VI
TABLE OF CONTENTS	VII
LIST OF TABLES	XI
LIST OF FIGURES	XIV
LIST OF ABBREVIATIONS	XV
1. LITERATURE REVIEW	1
1.1 INTRODUCTION	1
1.2 PRODUCTION OF ETHANOL IN NORTH AMERICA	1
1.2.1 Milling processes for ethanol production	4
1.2.2 Saccharification and fermentation processes	6
1.2.3 Distillation	7
1.2.4 Processing of by-products	8
1.3 THE USE OF BY-PRODUCTS FROM ETHANOL INDUSTRY IN LIVESTOCK RATINGS	8
1.3.1 The nutritional value of by-products from ethanol production for swine	10
1.4 CHEMICAL AND PHYSICAL CHARACTERISTICS OF DDGS	10
1.4.1 Energy content and digestibility	13
1.4.2 Protein and amino acid content and digestibility	14
1.4.3 Phosphorus content and availability	15
1.5 THE USE OF DISTILLER'S DRIED GRAINS WITH SOLUBLES IN SWINE INDUSTRY	16
1.5.1 Effect on voluntary feed intake	20

1.5.2 Effect on growth performance	20
1.5.3 Impact on nutrient excretion	22
1.6 POTENTIAL TO ENHANCE THE NUTRITIONAL VALUE OF DISTILLER’S DRIED GRAINS WITH SOLUBLES	24
1.6.1 The use of exogenous enzymes in livestock industry	24
1.6.2 Non-starch polysaccharides	24
1.6.3 Carbohydrase supplementation of livestock rations	26
1.6.4 Carbohydrase supplementation of swine rations.....	27
1.6.5 Xylanase supplementation of swine rations	30
1.6.5.1 Effect on energy digestibility	32
1.6.5.2 Effect on protein and amino acid digestibility	33
1.6.5.3 Effect on dry matter digestibility	35
1.6.5.4 Effect on performance.....	36
1.7 SUMMARY	38
2. NUTRITIONAL VALUE OF WHEAT AND CORN DISTILLER’S DRIED GRAIN WITH SOLUBLES: DIGESTIBILITY AND DIGESTIBLE CONTENTS OF ENERGY, AMINO ACIDS AND PHOSPHORUS, NUTRIENT EXCRETION AND GROWTH PERFORMANCE OF GROWER-FINISHER PIGS	41
2.1 ABSTRACT.....	41
2.2 INTRODUCTION.....	42
2.3 MATERIALS AND METHODS	44
2.3.1 Ingredients.....	44
2.3.2 Experimental Protocol.....	44
2.3.3 Chemical Analyses.....	49

2.3.4 Statistical analyses	50
2.4 RESULTS.....	51
2.4.1 Chemical characteristics	51
2.4.2 Nutrient digestibility and content.....	54
2.4.3 Nutrient excretion.....	58
2.4.4 Growth performance	62
2.5 DISCUSSION.....	64
3. EFFECT OF XYLANASE SUPPLEMENTATION OF WHEAT DISTILLER'S DRIED GRAINS WITH SOLUBLES ON ENERGY, AMINO ACID AND PHOSPHORUS DIGESTIBILITY AND DIGESTIBLE CONTENTS, AND NUTRIENT EXCRETION IN GROWER-FINISHER PIGS	72
3.1 ABSTRACT.....	72
3.2 INTRODUCTION.....	73
3.3 MATERIALS AND METHODS.....	75
3.3.1 Ingredients.....	75
3.3.2 Experimental Protocol.....	75
3.3.3 Chemical Analyses.....	78
3.3.4 Statistical analyses	80
3.4 RESULTS.....	80
3.4.1 Chemical characteristics	80
3.4.2 Nutrient digestibility and content.....	83
3.4.3 Nutrient excretion.....	89
3.5 DISCUSSION.....	93
4. GENERAL DISCUSSION.....	102

4.1 NUTRITIONAL CHARACTERISTICS OF DISTILLER’S DRIED GRAINS WITH SOLUBLES.	102
4.2 EFFECT OF XYLANASE SUPPLEMENTATION ON NUTRITIONAL CHARACTERISTICS OF WHEAT DISTILLER’S DRIED GRAINS WITH SOLUBLES	103
4.3 LIMITATIONS TO THE PRESENT STUDIES	104
4.4 FUTURE RESEARCH	105
4.5 IMPLICATIONS AND CONCLUSION	106
REFERENCES.....	108

LIST OF TABLES

Table 1.1 Starch content and ethanol yield of various feedstocks	3
Table 1.2 Chemical composition of corn and wheat based thin stillage and distiller's grains relative to original cereal grain (% DM)	9
Table 1.3 Approximate composition of brewer's (<i>Saccharomyces</i>) yeast	12
Table 1.4 Effect of graded levels of corn distiller's dried grains with solubles in corn- soybean meal diets on growth performance of starter pigs.....	17
Table 1.5 Effect of graded levels of corn distiller's dried grains with solubles on growth performance of grower-finisher pigs.....	18
Table 1.6 Effect of different levels of corn distiller's dried grains with solubles on reproductive performance of sows	19
Table 1.7 Effect of carbohydrases on nutrient digestibility (%) in different age groups of pigs	28
Table 1.8 Effect of carbohydrases on growth performance in different age groups of pigs	29
Table 2.1 Composition of experimental diets used in the digestibility study	45
Table 2.2 Composition of experimental diets used in the performance study	48
Table 2.3 Chemical characteristics of wheat, and corn, wheat+corn, and wheat distiller's dried grains with solubles (% DM)	52
Table 2.4 Phytate and P profile of wheat, and corn, wheat+corn, and wheat distiller's dried grains with solubles (% DM)	53
Table 2.5 Non-starch polysaccharide profile of wheat, and corn, wheat+corn, and wheat distiller's dried grains with solubles (% DM)	55

Table 2.6 Apparent ileal digestibility of energy and amino acids and total tract digestibility of energy and P in wheat, and corn, wheat+corn, and wheat distiller's dried grains with solubles	56
Table 2.7 Standardized ileal digestibility (%) of amino acids in wheat, and corn, wheat+corn, and wheat distiller's dried grains with solubles	57
Table 2.8 Apparent ileal digestible amino acid content (% DM), ileal and total tract digestible energy (kcal kg ⁻¹ DM) and total tract digestible P (% DM) contents in wheat, and corn, wheat+corn, and wheat distiller's dried grains with solubles.....	59
Table 2.9 Standardized ileal digestible amino acid content (% DM) in wheat and corn, wheat+corn, and wheat distiller's dried grains with solubles	60
Table 2.10 Effect of distiller's dried grains with solubles on dry matter, N and P intake, excretion and retention (on DM basis).....	61
Table 2.11 Growth performance of pigs fed diets containing wheat, or corn, wheat+corn, and wheat distiller's dried grains with solubles	63
Table 3.1 Composition of experimental diets	77
Table 3.2 Chemical characteristics of wheat distiller's dried grains with solubles, wheat used for the wheat distiller's dried grains with solubles and wheat in the basal diet (% DM)	81
Table 3.3 Phytate and P profile of wheat distiller's dried grains with solubles, wheat used for the wheat distiller's dried grains with solubles and wheat in the basal diet (% DM)	82

Table 3.4 Non-starch polysaccharide profile of wheat distiller's dried grains with solubles, wheat used for the wheat distiller's dried grains with solubles and wheat in the basal diet (% DM)	84
Table 3.5 Effect of ingredient (wheat and wheat distiller's dried grains with solubles) and xylanase supplementation on apparent ileal digestibility of energy and total tract digestibility of energy and phosphorus	85
Table 3.6 Effect of ingredient (wheat and wheat distiller's dried grains with solubles) and xylanase supplementation on apparent ileal amino acid digestibility (%)	86
Table 3.7 Effect of ingredient (wheat and wheat distiller's dried grains with solubles) and xylanase supplementation on standardized ileal amino acid digestibility (%).....	87
Table 3.8 Effect of ingredient (wheat and wheat distiller's dried grains with solubles) and xylanase supplementation on contents of apparent ileal digestible amino acid (% DM), ileal and total tract digestible energy (kcal kg ⁻¹ DM) and total tract digestible phosphorus (% DM)	90
Table 3.9 Effect of ingredient (wheat and wheat distiller's dried grains with solubles) and xylanase supplementation on standardized ileal digestible amino acid content (% DM).....	91
Table 3.10 Effect of ingredient (wheat and wheat distiller's dried grains with solubles) and xylanase supplementation on dry matter, nitrogen and phosphorus intake, excretion and retention (on DM basis).....	92

LIST OF FIGURES

Figure 1.1	Dry milling ethanol production process	5
Figure 3.1	Apparent and standardized ileal digestibility (%) of amino acids for individual dietary treatments.....	88

LIST OF ABBREVIATIONS

AA	amino acid
ADF	acid detergent fibre
ADFI	average daily feed intake
ADG	average daily gain
AID	apparent ileal digestibility
ANF	anti-nutritional factors
BW	body weight
CP	crude protein
CPS	Canada western prairie spring
DDG	distiller's dried grains
DDGS	distiller's dried grains with solubles
DDS	distiller's dried solubles
DE	digestible energy
DM	dry matter
GE	gross energy
he	hectare
IP	inositol phosphate
ME	metabolizable energy
MT	metric tonne
NDF	neutral detergent fibre
NEAA	non-essential amino acid
NPN	non-protein N
NSP	non-starch polysaccharide
SEM	standard error of the mean
SID	standardized ileal digestibility
U	unit
VFI	voluntary feed intake

1. LITERATURE REVIEW

1.1 Introduction

Widespread interest exists in many regions of the world, particularly in the United States, in producing ethanol from cereal grains for use as a fuel additive. The ethanol industry is therefore expanding rapidly. The trend of increased ethanol production is moving into Canada as well [Agriculture and Agri-Food Canada (AAFC) 2004]. Escalating fuel prices, comparatively low grain prices, opportunities for rural development, and environmental concerns can be considered as the major reasons for this interest. Furthermore, cereal grains are renewable energy sources while fuel produced from crude oil is not (Lipinsky 1981). Therefore, the abundant grain supplies in North America and diminishing fuel resources can be other reasons behind this interest. Finally, the quantity of ethanol produced by the fermentation of cereal grains continues to increase due to the high demand for a cleaner bio-based fuel additive. Consequently, increasing quantities of by-products are generated by the ethanol industry for which markets need to be developed. The feed industry is a logical first choice to market these by-products.

1.2 Production of ethanol in North America

Ethanol production from cereal grains basically involves the conversion of starch into simple sugars through enzymatic hydrolysis and subsequent fermentation of the sugars into ethanol and CO₂ using the yeast *Saccharomyces sp.*

A variety of feedstocks can be fermented to produce ethanol. These feedstocks vary in the content of starch and thereby vary in potential ethanol yield (Table 1.1). The usual feedstocks include corn, wheat, barley, rye, and sorghum. Corn is the most common feedstock for ethanol production in North America due to its abundance and greater yield of ethanol compared to the other cereal grains (Aines et al. 1987). However, cultivation of corn for seed production is not possible in some regions of Canada (AAFC 2004). Thus, wheat is the main feedstock for bio-ethanol production in the Canadian prairies (Beaulieu and Goodyear 1985; Warren et al. 1994). Similarly, ethanol production in Europe and Australia also depends on wheat (Hunwick 1980; Swinnen et al. 1988).

Compared to corn, wheat is less efficient as a feedstock. Grain represents 60 to 70% of ethanol production cost (Warren et al. 1994). Using wheat for fermentation, the ethanol yield was 340 litres MT^{-1} of feedstock or 510 to 710 litre $\text{ha}^{-1} \text{ year}^{-1}$ (Dale 1991). Depending on the cultivar of wheat fermented in commercial ethanol production, the yield ranged from 342 to 364 litre MT^{-1} of wheat (Sosulski and Sosulski 1994). Canada western prairie spring (CPS) wheat is preferred to hard red spring wheat, because CPS wheat contains more starch and less protein (Lee et al. 1991) and might result in higher ethanol yields, e.g., 396 litre MT^{-1} with a resultant by-product stream of 266 kg MT^{-1} , on dry matter basis (Beaulieu and Goodyear 1985).

Table 1.1 Starch content and ethanol yield of various feedstocks

Feedstock	Moisture (%)	Starch (%)	Ethanol Yield (L MT ⁻¹)
Starch	-	100.0	720
Sugar	-	-	654
Barley	9.7	67.1	399
Corn	13.8	71.8	408
Oats	10.9	44.7	262
Wheat	10.9	63.8	375

Source: Saskatchewan Agriculture and Food (1993)

1.2.1 Milling processes for ethanol production

Two main milling procedures are employed in the production of ethanol from feedstock, wet milling and dry milling, followed by five basic steps within each procedure: feedstock handling and processing, saccharification, fermentation, distillation and, recovery and processing of by-products (NAS-NRC, 1981).

In the wet milling process, the grain is initially steeped by adding water to maximize the amount of starch removed from the kernel. In addition to water, sulphur dioxide is added in corn wet milling process to soften the grain to split the kernel, separating the starch fraction from oil and protein components, thereby optimizing fermentation efficiency. In wheat wet milling, the bran and germ portions are eliminated by dry milling in a flourmill prior to steep in water. Despite lower ethanol output and other economic drawbacks, the wet milling process dominates the ethanol industry due to the yield of purer starch and higher value by-products (Kane and Reilly 1989; Rendleman and Hohmann 1993; Chang et al. 1995).

In the dry milling process, the starch fraction of the cereal grain is not separated (Rao 1979, Mulligan 1993); thus, the entire kernel of the grain is subjected to fermentation. Prior to water addition, the feedstock is cleaned and ground to reduce particle size, to facilitate enzymatic hydrolysis of starch to simple sugars. The entire dry milling process is outlined in Figure 1.1. The dry milling process is less popular than the wet milling process that represents approximately 40% of the market (Rendleman and Hohmann 1993). The dry milling process may produce a lower quality by-products, but has a cost advantage compared to wet milling.

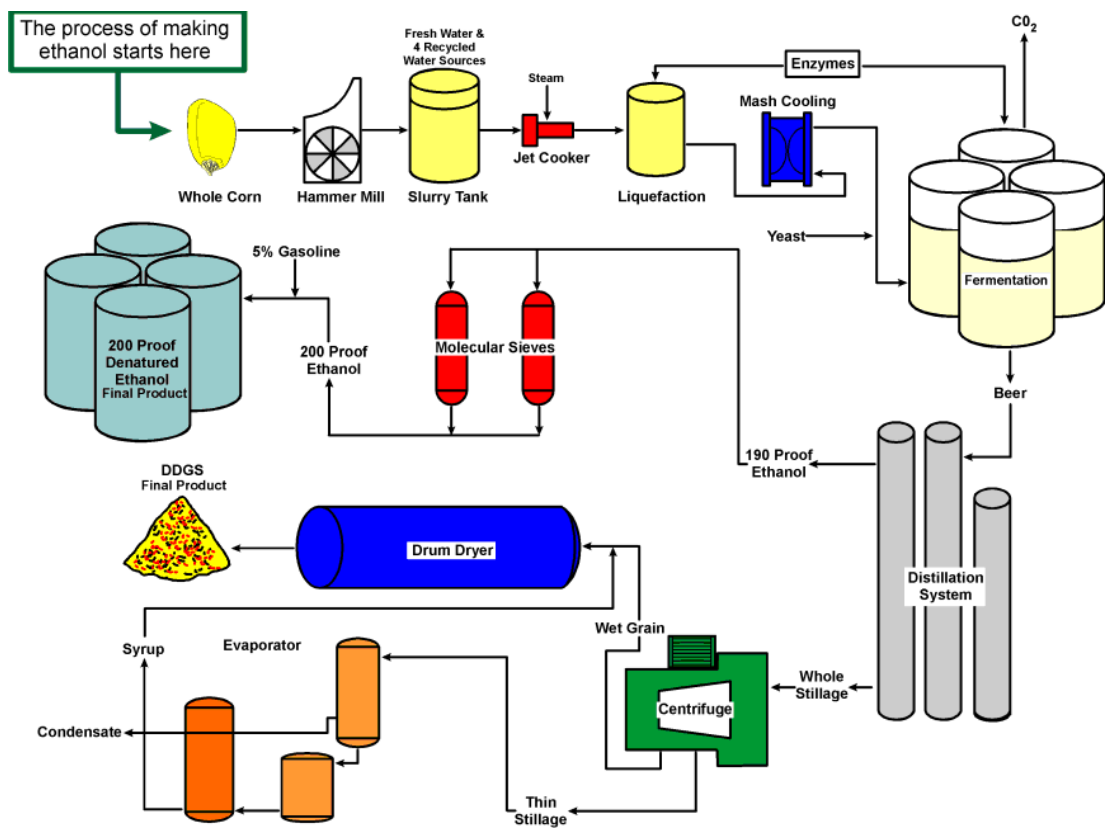


Figure 1.1 Dry milling ethanol production process
<http://www.exolmn.com/process.htm>

1.2.2 Saccharification and fermentation processes

In saccharification, the starch portion of feedstock is prepared for yeast fermentation, converting the starch into simple sugars. Following water addition, enzymes are introduced to start the saccharification process. The saccharification process is 70 to 80% effective, regardless of the duration that the enzymes are allowed to act upon the grain starch (Weigel et al. 1997). The aim of saccharification is to obtain a solution containing 14 to 20% sugar (NAS-NRC, 1981).

The fermentation process begins with the introduction of selected yeasts. For fermentation, *Sacchromyces cerevisiae* is mainly used owing to their quick, efficient production of ethanol and their ability to survive in high alcohol concentrations (Weigel et al. 1997). One molecule of simple sugar yields two molecules of ethanol and two molecules of CO₂ during the fermentation process.

Two major methods of fermentation exist: batch and continuous. Using batch fermentation, a single batch of the mixture is fermented at one time and another batch cannot be started until the first batch is completed. In contrast, continuous fermentation involves a continuous flow of fermentation, upon constant addition of feedstock to the system. Batch fermentation is commonly used due to the ease of operation, low capital cost and the relatively easy processing technique used. On the other hand, continuous fermentation has a high production capacity but the process control is more complex (Ingledew 1993).

1.2.3 Distillation

The distillation process involves the separation of ethanol from the ethanol-water mixture by vaporizing ethanol at 78°C. The maximum ethanol concentration that is usually achieved through this process is 96%.

The remaining product is called whole stillage (McCurdy 1986), which contains everything that was not fermented and converted to ethanol and CO₂. Due to the utilization of the starch portion of the grain during the fermentation process, remaining nutrients such as protein, fat, fibre, vitamins and minerals undergo a three-fold concentration in whole stillage on dry matter basis (Newland and Mahan 1990; Mustafa et al. 1999). The dry matter content of the whole stillage is 5 to 7% and, the amount of dry matter depends on the type of grain used for fermentation, water-to-grain ratio, quantity and type of back set stillage used, the fermentation process, and the efficiency of utilization of sugar during fermentation (Salonek 1995). On a volume basis, the ratio between ethanol yield and whole stillage output is 1 : 10 to 15 (Maiorella et al. 1983).

The whole stillage can be centrifuged or screened to fractionate into two by-products in almost equal quantities, wet distiller's grains and thin stillage (AAFC 2004). These by-products can be marketed in wet form or as dried products after drying (Larson et al. 1993; Ham et al. 1994). The centrifuged solid portion contains 25 to 35% dry matter, and can be dried as distiller's dried grains (DDG; Salonek 1995). The thin stillage represents the soluble portion and contains 4 to 8% dry matter, based on the feedstock used (Ham et al. 1994; Mustafa et al. 1998). This portion can be condensed and dried by evaporation to obtain distiller's dried solubles (DDS).

1.2.4 Processing of by-products

The DDG and DDS can be marketed and included in livestock rations separately or as a combined product: distiller's dried grains with solubles (DDGS). Therefore, by-products from ethanol production are traditionally divided into three categories: DDG, DDS and DDGS (Newland and Mahan 1990). However, DDGS is presently the primary by-product and the production of the other two types is minimal. At least 75% of the solids of the resultant whole stillage should be condensed and dried for the product to be accepted as DDGS (Association of American Feed Control Officials). The annual production of DDGS in North America is approximately 3.2 to 3.5 million MT (Shurson et al. 2000). Corn and wheat thin stillage and distiller's grains have a different chemical composition (Table 1.2).

1.3 The use of by-products from ethanol industry in livestock rations

The nutritive value of by-products from ethanol production in dairy and beef cattle industry is well documented and has been reported for a number of by-products originated from a variety of feedstocks including corn (Larson et al. 1993; Ham et al. 1994; Lodge et al. 1997ab; Al-Suwaiegh et al. 2002), wheat (Fisher et al. 1999; Iwanchysko et al. 1999; Mustafa et al. 1999 & 2000), sorghum (Lodge et al. 1997a), barley (Mustafa et al. 1999 & 2000ab), and rye and triticale (Mustafa et al. 2000b). The nutritional value for cattle has been evaluated using a variety of ethanol by-products including wet distiller's grains, thin stillage, DDG and DDGS.

Few studies have been conducted to investigate the nutritional value of by-products from ethanol industry for other species such as poultry (Potter 1966; Manley et al. 1978; Waldroup et al. 1981; Grizzle et al. 1982; Parsons et al. 1983; Cromwell et al.

Table 1.2 Chemical composition of corn and wheat based thin stillage and distiller's grains relative to original cereal grain (% DM)

Variable	Corn			Wheat		
	Grain	Thin stillage	Distiller's grains	Grain	Thin stillage	Distiller's grains
Crude protein	9-10	19.0	30.0	16.0	46.0	26.0
Crude fat	3-5	9.0	10.0	2.0	14.0	4.0
Ash	1-2	7.0	5.0	2.0	8.0	4.0
Acid detergent fibre	3.1	8.2	18.6	3.0	4.0	22.0
Neutral detergent fibre	10.8	27.0	43.0	16.0	34.0	74.0
Starch	70.0	25.0	8.0	63.0	2.0	2.0

Source: Mustafa et al. (2000) and NRC (1998)

1993; Hunt et al. 1997; Noll et al. 2001; Martinez et al. 2004) and fish (Cheng and Hardy 2004; Coyle et al. 2004). More than 80% of DDGS is included in ruminant diets, while less than 1% of the total annual production is used in swine diets (Shurson et al. 2000), although the latter inclusion portion is increasing rapidly.

1.3.1 The nutritional value of by-products from ethanol production for swine

In the early 1940's, the vitamin-rich DDS portion was the predominant by-product used in swine diets to supply B-vitamins and unidentified growth factors (Fairbanks et al. 1944). Currently, DDGS is the major distiller's feed used in swine diet formulations, representing over 95% of ethanol by-products (Newland and Mahan 1990).

However, the potential use of distiller's feeds in swine industry is not fully realized because of the scarcity of information on its nutritional value for swine. Product variability and lack of information about the nutritional value of DDGS from different sources are primary reasons for the reluctance of using corn DDGS in swine diets. Other concerns include, low AA digestibility (Wahlstrom et al. 1970) and an AA profile that is not well suited for pigs (Cromwell et al. 1983).

1.4 Chemical and physical characteristics of DDGS

Generally, the characteristics of DDGS depend mainly upon the type and quality of the cereal grain used for ethanol production. The nutrient composition of DDGS reflects the nutrient content of original cereal grain, with a higher concentration of the remaining nutrient following starch removal (Sosulski and Tarasoff 1997; Weigel et al. 1997; Mustafa et al. 1999). In addition, yeast biomass used for fermentation contributes at least 3.9% of the weight of the DDGS (Ingledew 1999). The composition of brewer's yeast

(*Saccharomyces*) indicates that yeast may contribute a considerable portion of the nutrients present in DDGS (Table 1.3). For instance, at least 5.3% of DDGS protein is provided by the yeast protein (Ingledew 1999).

Due to the diversity of feedstocks that can be fermented to produce ethanol and the variation in nutrient composition within grain sources, nutrient levels of DDGS have been highly variable (Spiehs 2001). The nutrient variability of DDGS cannot be entirely avoided, because DDGS is a by-product of a process primarily aimed at ethanol production. The variability for any by-product is thus associated with DDGS as well. Conversely, DDGS is a combination of DDG and DDS, two by-products with different nutrient profiles (Table 1.2). Thus, any variation in each of the two by-products will affect nutrient composition of DDGS. As a result, nutrient variability is wider in DDGS than other cereal by-products. Method of fermentation, completeness or duration of the fermentation process, drying temperature and duration, and the ratio of DDS blended with DDG are other major factors associated with nutrient variability of DDGS (Carpenter 1970; Olentine 1986; Spiehs et al. 2002). Annual and regional differences in the cereal grain used for fermentation process appear to be a major reason for variation within processing plants. The nutrient composition of corn samples cultivated in the same region in five continuous years (from 1997 to 2001) and of DDGS produced from respective corn samples different significantly among these years (Belyea et al. 2004).

Colour and odour of DDGS are two important physical characteristics related to nutritional value, because corn DDGS with golden yellow colour and sweet and slightly fermented odour is considered good quality while DDGS with dark brown colour and burnt or smoky odour is considered poor quality. Colour and odour are correlated with

Table 1.3 Approximate composition of brewer's (*Saccharomyces*) yeast

Variable	Content (g kg ⁻¹ dry yeast)
Carbohydrate	390 – 600
Protein	370 – 420
Ash	73 – 81
Phosphorus	14 – 20
Potassium	17
Sodium	0.7
Calcium	1.3
Iron	0.1
Magnesium	2.3
Cobalt	0.0002
Copper	0.033
Manganese	0.0057
Zinc	0.0387
Pantothenate	0.110 – 0.120
Choline	3.80 – 4.55
Thiamin (Vitamin B ₁)	0.092 – 0.150
Riboflavin (Vitamin B ₂)	0.035 – 0.045
Nicotinic acid/Niacin (NAD)	0.450
Pyridoxine (Vitamin B ₆)	0.043 – 0.050
Biotin	0.001
Inositol	3.9 – 5.0
Folic acid	0.010

Source: Ingledew (1999)

the nutritional properties of DDGS for monogastric animals (Cromwell et al. 1993).

1.4.1 Energy content and digestibility

Distiller's dried grains with solubles can be considered as a potential source of energy in livestock rations, based on its gross energy (GE) content. Nevertheless, this concept might be different for monogastrics due to the relatively high fibre content in DDGS, which may have limited the use of DDGS as an energy source in swine diets. However, the counteracting effect of a high fat content in DDGS results in a higher digestible energy (DE) value compared to that of the original cereal grain (Newland and Mahan 1990). The high fat content especially in corn DDGS, is associated with a higher gross energy content and therefore a higher DE content (Spiehs et al. 2002). The energy content varied considerably with the source and inclusion level of DDGS. The nutrient profile of corn DDGS originating from ten different ethanol plants varied widely in DE and metabolizable energy (ME) content (Spiehs et al. 2002). Specifically, DE content ranged from 3879 to 4084 kcal/kg and ME content ranged from 3639 to 3838 kcal/kg. Using three different inclusion levels of the same corn DDGS sample (10, 20 and 30%), grower-finisher pigs revealed the variability of DE and ME content (Spiehs et al. 2000). Specifically, the DE content ranged from 2830 to 5862 kcal/kg and the ME content ranged from 2551 to 5827 kcal/kg, indicating the difficulty of using book values for DE and ME content of DDGS in swine ration formulation.

The variability in DE and ME content of DDGS among different sources and inclusion rates is partially a reflection from variability in energy digestibility. The non-starch polysaccharides (NSP) are concentrated with other nutrients in DDGS after most of the starch in the cereal grain is removed during fermentation (Mustafa et al. 1999).

Increased NSP content in DDGS may reduce energy digestibility because the NSP fractions may impair digestion by disrupting the action of digestive enzymes and affecting intestinal microbial activity (van Barneveld et al. 1995; Grieshop et al. 2001).

1.4.2 Protein and amino acid content and digestibility

Few studies have been conducted during the last few years using corn DDGS in swine nutrition. Therefore, currently available AA composition and digestibility values are mainly based on studies conducted by Wahlstrom et al. (1980), Cromwell et al. (1983), and Cromwell and Stahly (1986).

Based on the relatively high crude protein content, DDGS can be considered as a potential source of supplemental protein and AA for swine. Nevertheless, the protein quality of DDGS is low due to deficiency of several indispensable AA, including lysine and tryptophan (Newland and Mahan 1990). Furthermore, corn DDGS samples originating from various sources may vary considerably in crude protein and AA composition (Cromwell et al. 1993; Spiehs et al. 2002). Initially, lysine was the most variable of the eleven AA analysed in the nine samples (Cromwell et al. 1993). Likewise a decade later, of the ten indispensable AA analyzed in the ten samples from different ethanol plants, lysine was the most variable, with a CV ranging from 2.9 to 25.7%, followed by methionine (Spiehs et al. 2002). Lysine and Methionine contents varied from 0.72 to 1.02 and from 0.49 to 0.69% (dry matter basis), respectively.

The AA profile of DDGS is not well-suited for pigs (Cromwell et al. 1983). The lack of lysine, the first limiting AA in swine nutrition, is particularly critical. Some of the lysine is destroyed during the fermentation and/or drying processes of DDGS. Following overheating, lysine forms complexes with carbonyl groups, the Maillard

reaction, which are associated with a colour change and stimulated by heat in the presence of moisture, the exact conditions present in the drying process (Patience et al. 1995). The relatively low total lysine content in DDGS suggests the occurrence of similar type processes with overheating of DDGS samples.

The DDGS has a lower AA digestibility than cereal ingredients in swine (Wahlstrom et al. 1970). Similar to energy digestibility, the NSP fraction of DDGS may affect the digestion process of AA as well (van Barneveld et al. 1995; Groeshop et al. 2001). The negative effects of NSP on DM, energy and AA digestibility have been well described (Kennelly and Aherne 1980; Fairbairn et al. 1999; Zijlstra et al. 1999; de Lange 2000; de Lange et al. 2000; Jondreville et al. 2001; Yin et al. 2002). Based on the high fibre content of DDGS, plausible factors contributing to low AA digestibility in DDGS could be: decreased enzymatic breakdown of dietary protein, thereby reduced absorption and increased undigested protein (AA) in digesta, increased endogenous AA losses (Jondreville et al. 2001) and binding of AA and peptides to fibre (Bergner et al. 1981, as referenced by Lenis et al. 1996).

1.4.3 Phosphorus content and availability

The bioavailability of P was enhanced in DDGS due to fermentation during which phytate-P was hydrolyzed by microbial phytase (Singsen et al. 1972 as referenced by Cromwell 1979). Approximately 60 to 70% of the total P in a cereal-based diet is bound to phytate, and this phytate-P is unavailable to the pig (Baker 1991). The indigestible P is excreted in faeces, and may impact the environment negatively if not managed properly (Cromwell et al. 1993; Liu et al 1998; NRC 1998). On the other hand, P is a mineral of particular interest because it is the third most expensive nutrient after energy

and protein (NRC 1998). A higher content and digestibility of P in DDGS compared to cereals may indicate environmental and economical benefits of DDGS for the management of P in swine diets and manure.

1.5 The use of distiller's dried grains with solubles in swine industry

The use of corn DDGS in swine rations has been tested with different inclusion rates in pigs of different growth stages including starter pigs (Combs and Wallace 1969; Richards 1979; Wahlstrom and Libal 1980; Orr et al. 1981; Cromwell et al. 1985; Miller et al. 1987), grower-finisher pigs (Livingstone and Livingston 1966; Wahlstrom and German 1968; Walstrom and Libal 1969; Wahlstrom et al. 1970; Combs and Wallace 1970; Smelski and Stothers 1970; Harmon 1975; Cromwell et al. 1983; Cromwell et al. 1984; Cromwell et al. 1993), and breeding stock (Thong et al. 1978). Some studies evaluated the AA profile of DDGS and investigated the effect of lysine supplementation on performance of pigs fed DDGS incorporated diets (Wahlstrom et al. 1970; Cromwell et al. 1983; Cromwell and Stahly 1986). Possibilities of alleviating the negative effects associated with DDGS on growth performance of weanling pigs were studied by adding antibiotics to the diet and observed better performance with diets with antibiotics (Cromwell et al. 1985). More recently, description of the nutritional value of corn DDGS for swine has gained renewed attention (Shurson et al. 2000; Spiehs 2001; Spiehs et al. 2002; Whitney and Shurson 2004). Nevertheless, information on the use of wheat DDGS in swine nutrition remains scarce. The effects of different levels of corn DDGS on growth performance of pigs in different growth stages are summarized in Tables 1.4, 1.5 and 1.6.

Table 1.4 Effect of graded levels of corn distiller's dried grains with solubles in corn-soybean meal diets on growth performance of starter pigs

Study	DDGS (%)	ADG (kg)		ADFI (kg)		Feed Efficiency	
		Control	DDGS	Control	DDGS	Control	DDGS
Combs and Wallace 1969	10	0.58	0.61	1.25	1.39	2.16	2.26
	20	0.58	0.60	1.25	1.37	2.16	2.29
Wahlstrom and Libal 1980	10	0.35	0.34	0.61	0.61	1.75	1.82
	20	0.35	0.32	0.61	0.58	1.75	1.84
	30	0.35	0.29	0.61	0.56	1.75	1.90
Orr et al. 1981	2.5	0.27	0.30	0.47	0.50	1.74	1.65
	5.0	0.27	0.27	0.47	0.46	1.74	1.74
Cromwell et al. 1985	10	0.63	0.60	1.26	1.28	2.01	2.13
	10*	0.63	0.66	1.27	1.35	1.99	2.05

* experimental diets with an antibiotic

Table 1.5 Effect of graded levels of corn distiller's dried grains with solubles on growth performance of grower-finisher pigs

Study	DDGS (%)	ADG (kg)		ADFI (kg)		Feed efficiency	
		Control	DDGS	Control	DDGS	Control	DDGS
Livingstone and	14.7	0.63	0.64	NR	NR	3.38	3.34
Livinston 1966 ^a	25.0	0.63	0.58	NR	NR	3.38	3.56
Wahlstrom et al. 1970 ^b	5.0	0.78	0.81	2.52	2.58	3.21	3.20
	10.0	0.78	0.78	2.52	2.46	3.21	3.17
	20.0	0.78	0.75	2.52	2.69	3.21	3.59
Wahlstrom et al. 1970 ^c	5.0	0.77	0.76	2.30	2.15	2.97	2.82
	10.0	0.77	0.77	2.30	2.11	2.97	2.73
	20.0	0.77	0.73	2.30	2.09	2.97	2.89
Combs and	10.0	0.83	0.82	2.66	2.85	3.22	3.48
Wallace 1970 ^b	20.0	0.83	0.86	2.66	3.02	3.22	3.56
Smelski and	7.5	0.75	0.74	2.75	2.70	3.71	3.65
Stothers 1972 ^d	15.0	0.75	0.70	2.75	2.70	3.71	3.86
Cromwell et al. 1983 ^b	5.0	0.80	0.88	2.69	2.87	3.35	3.28
	10.0	0.80	0.83	2.69	2.79	3.35	3.36
	20.0	0.80	0.79	2.69	2.74	3.35	3.47
	40.0	0.80	0.76	2.69	2.80	3.35	3.70
Cromwell et al. 1984 ^b	2.5	0.83	0.83	2.59	2.61	3.12	3.14
	5.0	0.83	0.83	2.59	2.59	3.12	3.12
	10.0	0.83	0.82	2.59	2.53	3.12	3.09

NR - not reported

^a Barley, wheat, soybean meal basal diet

^b Corn, soybean meal basal diet without synthetic lysine supplementation

^c Corn, soybean meal basal diet with synthetic lysine supplementation

^d Barley, soybean meal basal diet with synthetic lysine supplementation

Table 1.6 Effect of different levels of corn distiller's dried grains with solubles on reproductive performance of sows

Variable	Distiller's dried grain with solubles (%)		
	0	17.7	44.2
Live pigs born	8.8	8.6	8.2
Birth weight (kg)	1.4	1.4	1.4
Pigs weaned (28 d)	7.3	7.4	7.3
Weaning weight (kg)	6.5	6.7	6.6
Feed intake in lactation (kg)	123	105	112
Sow weight loss in lactation (kg)	5.3	3.9	1.4
Source, Thong et al. (1978)			

1.5.1 Effect on voluntary feed intake

Voluntary feed intake (VFI) may be reduced when corn DDGS is included in swine diets (Wahlstrom and Libal 1980; Whitney and Shurson 2004). Reasons for the reduction in VFI remain unclear and dietary inclusion of DDGS seemed to negatively affect palatability (Whitney and Shurson 2004), probably due to high fibre content. Factors, which are unfavourable for the palatability and feed intake, such as mold spores, can be concentrated in DDGS, during fermentation. Other possibilities causing a reduced VFI include a significant AA imbalance, including an increased level of non-essential AA [(NEAA) Henry et al. 1992; Henry 1995; Hahn et al. 1995] and high energy density due to relatively high fat content of DDGS (Azain 2001). An increased level of NSP will increase fermentation in the hind gut of pigs (Govers et al. 1999). Oligosaccharide fermentation may occur in the swine small intestine, but this dietary component is mostly fermented in the hindgut (Wigan et al. 1994). The hindgut distension caused by elevated fermentation and resultant increase in digesta retention time has been associated with the reduced VFI of pigs fed DDGS (Cherbut et al. 1988).

1.5.2 Effect on growth performance

The appropriate inclusion levels of DDGS at different growth stages have received considerable debate. In starter pigs, growth performance was unaffected at levels up to 10% DDGS in a basal diet of corn-soybean meal (Combs and Wallace 1969; Wahlstrom and Libal 1980), suppressed at the level of 10% DDGS in the diet (Cromwell et al. 1985), or increased numerically with 2.5% DDGS in a basal diet of corn-soybean meal without additional benefits at 5% DDGS (Orr et al. 1981). Nevertheless, DDGS was

incorporated successfully at levels up to 5% into starter pig feeding programs (Richards 1979).

The DDGS was incorporated as an alternate protein and energy source at levels up to 10% of grower-finisher pig diets without adversely affecting growth performance (Wahlstrom and German 1968; Cromwell et al. 1984; Cromwell et al. 1985). However, performance of grower-finisher pigs was variable at higher DDGS inclusion levels. In some studies, performance in grower-finisher pigs fed diets containing 20% DDGS was equal to the pigs fed corn-soybean meal basal diets (Harmon 1975; Cromwell et al. 1983). However, performance was reduced in grower-finisher pigs fed diets containing 20% DDGS in another study (Wahlstrom and Libal 1969). Higher inclusion levels of DDGS (30 and 40%) in the diets of grower-finisher pigs adversely affected the growth (Cromwell et al. 1983).

In sows, DDGS might have less negative effects on performance. Corn DDGS was included at levels up to 44.2% in a corn-soybean meal diet without affecting reproductive performance (Thong et al. 1978; Table 1.6). Litter size, average birth weight of pigs, number of pigs weaned per litter and average weaning weight of pigs did not differ among treatments. In addition, feed intake of lactation sows, body weight gains and losses of gestation and lactation sows, respectively, were similar among the dietary treatments.

Inclusion of DDGS increases dietary CP content with a simultaneous increase of NEAA, because DDGS is high in CP and has an improperly balanced AA profile. An increased supply of AA will increase AA absorption in excess of AA requirement for maintenance and protein synthesis. The excess AA are catabolized and the NH_3 generated as a result of deamination is eliminated from the body via the kidney as urea

in urine. Accordingly, feeding pigs diets containing DDGS increases N excretion, thereby increasing the requirement of metabolic energy for AA catabolism and urea synthesis, leaving less available energy to support production functions (Spiehs et al. 2002). On the other hand, the dietary lysine intake may be comparatively low for pigs fed diets containing DDGS, mainly due to lower feed intake and lower total lysine content in DDGS, which together with lower lysine availability of DDGS may constrain protein accretion of pigs. The relationship between lysine intake and growth performance of grower-finisher pigs is well-documented (Henry et al. 1988; Chiba et al. 1991; Friesen et al. 1994) and variability in growth performance of pigs fed DDGS incorporated diets can partly be attributed to the differences in lysine intake as well as availability.

1.5.3 Impact on nutrient excretion

Nitrogen intake will be relatively high in pigs fed DDGS diets owing to the higher crude protein content in DDGS. The increase in N intake will increase in N excretion in faeces and urine, and the improperly-balanced AA profile in DDGS may further increase the N excretion of pigs, because about 50% of N excretion in pig manure can be attributed to the poor AA balance in the diet (de Lange 2004).

Dietary fermentable fibre may shift N excretion from urea in urine to bacterial protein in faeces (Morgan and Whittemore 1988; Mroz et al. 1993; Lenis et al. 1996; Canh et al. 1997; Canh et al. 1998; Zervas and Zijlstra 2002). The bacterial N assimilation with the presence of fermentable NSP in the hindgut is the underlying reason for this phenomenon. Therefore, a shift of N excretion from urine to faeces can be expected with the inclusion of DDGS in the diet due to the concentrated NSP fraction of DDGS. In addition, reduced AA digestibility caused by the NSP can be considered as

another factor associated with N excretion patterns. Environmentally, faecal N is preferable to urinary N, because faecal N (mainly bacterial protein) is less susceptible to rapid decomposition than urinary N (mainly urea), which is easily converted into ammonium and CO₂ by faecal urease (Canh et al. 1998b; Mroz et al. 2000).

The content of non-phytate P is higher in DDGS than in the unfermented starting material due to the fermentation during which phytate-P was hydrolyzed by microbial phytase (Singsen et al. 1972: as referenced by Cromwell 1979), and consequently P digestibility or availability is higher. Therefore, based on higher unbound P content in DDGS, a high intake of digestible P can be expected for pigs fed DDGS diets. Increased amount of P appears in faeces and urine, as a consequence of increased P intake (Cromwell et al. 1993; Jongbloed et al. 1993; Liu et al. 1998; Ekpe et al. 2002). The P excretion in swine mainly occurs in faeces, and increased P excretion in faeces with increasing levels of digestible P intake can be expected (Ekpe et al. 2002; Zhang et al. 2003). A portion of P excreted by pig is a direct result of feeding excessive levels of digestible P (Ekpe et al. 2002). Therefore, P excretion in the swine industry can be managed by dietary manipulation and supplemental inorganic P can be reduced when DDGS is incorporated into the diet. Reducing total P content may reduce feed costs, thereby increasing net income. The strategy indicates environmental and economical benefits of DDGS for the management of P in swine diets and manure.

1.6 Potential to enhance the nutritional value of distiller's dried grains with solubles

Many feed ingredients, including cereals and their by-products, contain considerable amounts of anti-nutritional factors (ANF), which affect the nutritional value of those ingredients when fed to monogastrics. In recent years, concerted efforts to enhance the nutritional value of such feedstuffs have been undertaken to maximize nutrient utilization. The use of exogenous enzymes has been recognized as one of the ways to improve nutrient utilization. Apart from improved nutrient utilization from feedstuffs, the quality of the environment might be improved by reducing the manure output and pollution associated with manure (Classen 1998).

1.6.1 The use of exogenous enzymes in livestock industry

Several possible mechanisms have been proposed to explain the beneficial effects of the use of exogenous enzymes in livestock industry: (1) removal of anti-nutritional factors (ANF); (2) increasing the digestibility of existing nutrients; (3) increasing the digestibility of NSP; and (4) supplementing host endogenous enzymes (Classen 1998). Nevertheless, the current primary objective of the use of exogenous enzymes in commercial livestock rations is the potential degradation of NSP or phytic acid to maximize the nutrient utilization from feedstuffs.

1.6.2 Non-starch polysaccharides

The cell walls of cereal grains are primarily comprised of complex carbohydrates referred to as NSP (Choct 1997), and consist predominantly of β -glucans in barley and oats, and arabinoxylan in wheat, rye and triticale (Englyst et al. 1989; Bach Knudsen

1997; Zijlstra et al. 1999). The β -glucans consist of linear chains of β -glycosyl residues linked by β (1-3) or (1-4) bonds with predominant (1-4) linkages (MacGregor and Fincher 1993). Arabinoxylans, on the other hand, are a β (1-4) linked polymers of the pentoses arabinose and xylose, and are hence referred to as pentosans (Fincher and Stone 1986).

The predominant NSP in cell walls varies among cereal grains. Furthermore, the content of NSP varies widely among cereals, with barley and hullless barley containing more NSP than wheat or corn (Fincher and Stone 1986). Finally, the NSP content varies among samples of barley (Fairbairn et al. 1999), wheat (Zijlstra et al. 1999) and hullless barley (Andersson et al. 1999). Not only grain type and cultivar but also the rate of fertilization and growing conditions may affect the NSP content within a crop year (Oscarsson et al. 1998).

The NSP fraction of cereal grains has drawn a considerable attention in poultry and swine ration formulation, because the digestive system of monogastrics lacks the appropriate endogenous enzymes required to hydrolyse NSP. In poultry, NSP associated digesta viscosity is mainly responsible for the reduced nutrient utilization and growth performance (Choct and Annison 1992; Almirall et al. 1995). Nevertheless, viscosity-altering properties of NSP are not as likely to be an issue of the same magnitude in swine nutrition (Bedford et al. 1992; Johansen et al. 1997; Pluske et al. 1999; Mavromichalis et al. 2000; Medel et al. 2002; Högberg and Lindberg 2004; Zijlstra et al. 2004). Rather, as a physical barrier, interferences of NSP with the action of digestive enzymes and digestion process are more important (van Barneveld et al. 1995; Grieshop et al. 2001). The negative correlation between the energy digestibility and the dietary

content of NSP is well-documented (Fairbairn et al. 1999; Zijlstra et al. 1999; de Lange et al. 2000; Yin et al. 2002).

In swine, apparent total-tract NSP digestibility is higher than apparent ileal digestibility, due to microbial degradation in the hindgut (Li et al. 1996). From a nutrient utilization point of view, however, nutrients absorbed after fermentation in the large intestine have less nutritional value for pigs than nutrients absorbed in the small intestine after digestion with endogenous digestive enzymes (Noblet et al. 1994; Fuller and Reeds 1998; Noblet 1999). Moreover, the net energy value of carbohydrates fermented and absorbed as volatile fatty acids in the large intestine of pig is 30% lower than that from the small intestine (Dierick et al. 1989).

1.6.3 Carbohydrase supplementation of livestock rations

Enzyme preparations have been formulated to facilitate the digestion process of NSP in monogastrics, prior to fermentation in the large intestine. These enzymes are commonly referred to as carbohydrases. The main carbohydrases used in livestock rations to alleviate the detrimental effects associated with NSP are xylanase and β -glucanase. The effects of carbohydrases on nutrient digestibility and growth performance at different growth stages of non-ruminants, using either xylanase or β -glucanase in poultry and swine diets have been studied in depth. In addition, both carbohydrases combined have been studied (Thacker et al. 1992a; Inborr et al. 1993; Yin et al. 2001a,b; Högberg and Lindberg 2004; Zijlstra et al. 2004; Thacker and Rossnagel 2005), making definitions of relationships between specific activities and beneficial effects difficult. Interestingly, the influence of these supplemental enzymes, mostly in combination with other fibrolytic enzymes, has also been studied in ruminants, despite the fibre degradation ability of

microorganisms in the rumen (ZoBell et al. 2000; Bowman et al. 2002; Kung et al. 2002; Colombatto et al. 2003; Nowak et al. 2003; Yu et al. 2005).

Experiments with swine however, often demonstrated inconsistent responses to xylanase supplementation, only some studies showing an improvement in nutrient digestibility (Rattay et al. 1998; Yin et al. 2000; Barrera et al. 2004) and growth performance (Van Lunen and Schulze 1996; Barrera et al. 2004), while the other experiments concluding with a lack of response either in nutrient digestibility (Thacker et al. 1991; Bedford et al. 1992; Mavromichalis et al. 2000; Yin et al. 2001b; Diebold et al. 2005) or performance (Bedford et al. 1992; Inberr et al. 1993; Mavromichalis et al. 2000).

1.6.4 Carbohydrase supplementation of swine rations

Numerous attempts have been made to maximize nutrient digestibility and hence improve the performance of pigs fed diets based on cereal grains supplemented with exogenous carbohydrases. Nevertheless, most of these studies have not been successful in demonstrating improvements in performance of a magnitude similar to those observed in poultry for either starter pigs (Inberr and Ogle 1988; Officer 1995; Thacker et al. 1992b; Inberr et al. 1993; Baidoo et al. 1998; Jensen et al. 1998; Li et al. 1999; Mavromichalis et al. 2000; Högberg and Lindberg 2004) or grower-finisher pigs (Newman et al. 1980, 1983; Thacker et al. 1989; Thacker et al. 1992a, 1992b; Baas and Thacker 1996; Thacker and Campbell 1999; Mavromichalis et al. 2000; Thacker and Rossnagel 2005). Effects of carbohydrases on nutrient digestibility and growth performance in different growth stages of pigs are summarized in Tables 1.7 and 1.8, respectively.

Table 1.7 Effect of carbohydrases on nutrient digestibility (%) in different age groups of pigs

Study	Dry matter		Energy		Crude protein	
	Control	Enzyme	Control	Enzyme	Control	Enzyme
<i>Starter pigs</i>						
Thacker et al. 1992b ^z	83.7	83.1	82.6	81.9	78.4	77.8
Baidoo et al. 1998 ^y						
Apparent ileal	56.0 ^b	64.5 ^a	57.1 ^b	63.3 ^a	57.6	61.8
Total tract	76.1	80.3	75.0	79.4	66.2	72.0
Mavromichalis et al. 2000 ^x	87.2	87.8	NR	NR	86.9	88.0
<i>Grower-finisher pigs</i>						
Thacker et al. 1992a ^v	75.8	76.4	74.2	75.6	72.2	73.7
Thacker et al. 1992a ^u	81.2	82.6	72.5	76.5	78.9	80.6
Thacker et al. 1992b ^t	76.2 ^b	80.6 ^a	76.3	79.3	78.6	75.3
Mavromichalis et al. 2000 ^x	84.7	86.0	NR	NR	81.9	84.8
Thacker and Rossnagel 2004 ^s	79.0	79.6	78.4	78.8	77.9	79.7
Thacker and Rossnagel 2004 ^r	72.5	74.7	72.8	74.8	79.1	81.4
Thacker and Rossnagel 2004 ^q	73.1	74.3	73.8	75.1	79.3	80.8

NR - not reported

^z Hulless barley - SBM based diets with β -glucanase

^y Hulless barley based diets with a combination of xylanase and β -glucanase

^x Wheat soybean meal based diets with xylanase

^v Barley - SBM based diets with a combination of xylanase and β -glucanase

^u Rye - SBM based diets with a combination of xylanase and β -glucanase

^t Barley - SBM based diets with β -glucanase

^s Barley - SBM based diets with a combination of xylanase and β -glucanase

^r Normal oat - SBM based diets with a combination of xylanase and β -glucanase

^q High fat oat - SBM based diets with a combination of xylanase and β -glucanase

Table 1.8 Effect of carbohydrases on growth performance in different age groups of pigs

Study	ADG (g)		ADFI (g)		Feed efficiency	
	Control	Enzyme	Control	Enzyme	Control	Enzyme
<i>Starter pigs</i>						
Thacker et al. 1992b ^z	254	274	534	547	2.20	2.03
Baidoo et al. 1998 ^y	462	511	651	700	1.41	1.37
Mavromichalis et al. 2000 ^x	450	470	520	570	1.15	1.22
Högberg and Lindberg 2004 ^w	195	198	295	304	1.51	1.53
<i>Grower-finisher pigs</i>						
Thacker et al. 1992a ^v	840	860	2140	2220	2.53	2.59
Thacker et al. 1992a ^u	740	710	1770	1750	2.40	2.48
Thacker et al. 1992b ^t	830	840	2560	2470	3.09	3.04
Baidoo et al. 1998 ^y	985	1025	2315	2122	2.35	2.07
Mavromichalis et al. 2000 ^x	900	930	2910	2990	3.23	3.23
Thacker and Rossnagel 2004 ^s	1020	1060	2670	2690	2.62	2.54
Thacker and Rossnagel 2004 ^r	1050	1030	2740	2770	2.60	2.70
Thacker and Rossnagel 2004 ^q	1060	1040	2710	2660	2.57	2.57

^z Hulless barley - SBM based diets with β -glucanase

^y Hulless barley based diets with a combination of xylanase and β -glucanase

^x Wheat soybean meal based diets with xylanase

^w Diets with a combination of xylanase and β -glucanase contained barley, oats, triticale, wheat and wheat bran

^v Barley - SBM based diets with a combination of xylanase and β -glucanase

^u Rye - SBM based diets with a combination of xylanase and β -glucanase

^t Barley - SBM based diets with β -glucanase

^s Barley - SBM based diets with a combination of xylanase and β -glucanase

^r Normal oat - SBM based diets with a combination of xylanase and β -glucanase

^q High fat oat - SBM based diets with a combination of xylanase and β -glucanase

Recently, the suppressive effect of NSP on VFI received attention. A positive quadratic dose-response correlation existed between ADFI and ADG upon carbohydrase supplementation of starter pig diets, indicating that carbohydrase will improve VFI, but suggest that an excess breakdown of the respective NSP in the gastro-intestinal tract may thereafter directly or indirectly inhibit voluntary feed intake (Zijlstra et al. 2004).

Supplemental carbohydrases have been tested for their ability to improve nutrient digestibility in swine. A number of previous studies reported no improvement in nutrient digestibility either in starter (Bedford et al. 1992; Thacker et al. 1992b; Li et al. 1996b; Mavromichalis et al. 2000; Högberg and Lindberg 2004; Zijlstra et al. 2004; Diebold et al. 2005) or grower-finisher pigs (Graham et al. 1986, 1989; Thacker et al. 1989; Thacker et al. 1992a, 1992b; Li et al. 1996a; Mavromichalis et al. 2000). Under normal conditions and with good quality cereal ingredients, beneficial effects of carbohydrases on nutrient digestibility and growth performance might therefore be difficult to obtain.

1.6.5 Xylanase supplementation of swine rations

A considerable amount of research with carbohydrases has been conducted using supplementary xylanase, which is generally used in wheat-based diets. Nevertheless, studies to evaluate the effect of supplementary xylanase on nutrient digestibility and performance in wheat-based diets for swine are limited and controversial.

While a number of studies reported a beneficial effects of xylanase on pig growth performance (Dierick 1989; Van Lunen and Schulze 1996; Barrera et al. 2004; Zijlstra et al. 2004), other studies reported no significant effects (Thacker et al. 1991, 1992a; Bedford et al. 1992; Inborr et al. 1993; Thacker and Bass 1996; Högberg and Lindberg 2004; Thacker and Rossnagel 2005). Inconsistent results have also been reported among

weight categories of swine. For example, xylanase supplementation did not affect performance in nursery pigs and in one of two experiments with finisher pigs fed wheat-based diets; however, xylanase improved performance in the second experiment with finisher pigs (Mavromichalis et al. 2000). Similarly, supplemental xylanase affected nutrient digestibility in pigs inconsistently. Sometimes xylanase improved digestibility (Graham et al. 1988; Baidoo et al. 1998; Rattay et al. 1998; Yin et al. 2000b; Barrera et al. 2004; Thacker and Rossnagel 2005), while nutrient digestibility of pigs also sometimes did not improve with supplementary xylanase (Thacker et al. 1991, 1992a; Bedford et al. 1992; Mavromichalis et al. 2000; Yin et al. 2001; Högberg and Lindberg 2004; Zijlstra et al. 2004; Diebold et al. 2005).

One unit (U) of xylanase activity is defined as the amount of enzyme required to release 1 mol of reducing sugars (expressed as xylose) in 1 min (Tervila-Wila et al. 1996). Xylanase supplementation at rates of 5500, 11,000 and 16,500 U kg⁻¹ improved nutrient digestibility and some performance variables linearly and quadratically in grower pigs fed wheat-based diets, with the best response with the intermediate inclusion level of xylanase (11,000 U kg⁻¹; Barrera et al. 2004). In another experiment conducted using finishing pigs fed wheat-based diets in mash and pellet forms supplemented with two different forms of xylanase, powder and liquid with 4000 and 8000 U g⁻¹ xylanase activity, respectively, xylanase improved growth performance and nutrient digestibility in pigs fed mash diets but not in pigs fed pelleted diets (Park et al. 2003). Inconsistency in growth performance and nutrient digestibility in pigs fed diets with supplementary xylanase can therefore be attributed to the factors such as growth stage of pigs, level of xylanase and the nature of the diet.

1.6.5.1 Effect on energy digestibility

Xylanase supplementation has improved energy digestibility in some studies, whereas the rest of the studies lacked a beneficial of response. The inconsistency can be attributed to variations among the major ingredients in NSP content, the level of supplementary xylanase and the age of experimental pigs.

Supplemental xylanase in diets based on wheat and its by-products improved ileal and total-tract apparent energy digestibility 3 and 1%, respectively, in grower pigs (Yin et al. 2000b). In weanling pigs, xylanase supplementation in combination with β -glucanase of diets based on hull-less barley improved both apparent ileal and total-tract digestibility of energy 11 and 6%, respectively (Baidoo et al. 1998) and of diets based on barley and pollard, improved the apparent ileal digestibility of energy 4%, without affecting total-tract digestibility (Graham et al. 1988). In addition, the same enzyme combination improved the total tract digestibility of energy in grower-finisher pigs fed diets containing normal or high fat oat (Thacker and Rossnagel 2005).

In contrast, xylanase supplementation on meal and pellet form rye-based diets did not improve total tract energy digestibility in weanling pigs (Thacker et al. 1991). Similarly, supplementing xylanase to diets based on hullless barley or wheat did not improve either apparent ileal or total tract energy digestibility in young or weaner pigs, respectively (Yin et al. 2001b; Diebold et al. 2005). Supplementing diets based on barley, wheat, oats, triticale and wheat bran with xylanase together with β -glucanase did not affect total tract digestibility of energy in weaned piglets (Högberg and Lindberg 2004). The same enzyme combination at different inclusion rates in a diet based on wheat and canola meal did not affect apparent digestibility of energy in each of the four segments of the small intestine examined in weaned pigs (Zijlstra et al. 2004).

Moreover, xylanase in combination with β -glucanase did not influence total tract energy digestibility of growing or finishing pigs fed diets based on barley or rye (Thacker et al. 1992a). Interestingly, xylanase together with β -glucanase did not affect the total tract energy digestibility in grower pigs fed diets containing barley, wheat, SBM and low-mucilage canola meal, but improved the digestibility coefficient in pigs fed the same diet, replacing low-mucilage canola meal with Candle canola meal (Bell and Keith 1991). Combined, the data indicate that effects of xylanase on energy digestibility are dose dependent and may depend on the amount of arabinoxylans in the diet and whether the arabinoxylans are indeed a factor limiting energy digestibility in the specific diet.

1.6.5.2 Effect on protein and amino acid digestibility

Beneficial effects of xylanase supplementation on protein and AA digestibility in swine have been observed. With the increase in the rate of xylanase supplementation, apparent ileal digestibility of CP and AA improved linearly and quadratically in grower pigs fed wheat-based diets, achieving the highest digestibility values in diets supplemented with 11,000 U g⁻¹ enzyme activity, improving CP digestibility 7% (Barrera et al. 2004). Supplementation of a diet containing barley, wheat, wheat bran and SBM with xylanase improved apparent ileal digestibility of CP in growing pigs 5% (Rattay et al. 1998). Furthermore, supplemental xylanase in diets based on wheat or its by-products improved apparent ileal digestibility of some of the indispensable AA and of CP 2% and total tract digestibility of CP 1% in grower pigs (Yin et al. 2000b). Supplementary xylanase in combination with β -glucanase in two diets, each containing one of two varieties of hull-less barley improved both apparent ileal and total tract digestibility of CP 8 and 9%,

respectively, and apparent ileal digestibility of all AA in both diets (Baidoo et al. 1998). In addition, supplementing xylanase together with β -glucanase, improved the apparent ileal digestibility of CP 7%, without affecting total tract digestibility, in weanling pigs fed diets based on barley and pollard (Graham et al. 1988). The same enzyme combination improved the total tract digestibility of CP in grower-finisher pigs fed diets containing normal or high fat oat (Thacker and Rossnagel 2005). However, supplementing xylanase to rye-based diets in meal and pellet forms did not improve the total tract CP digestibility in weanling pigs (Thacker et al. 1991). In addition, supplementary xylanase in rye-based diets did not show any improvement in CP (N) digestibility in weanling pigs (Bedford et al. 1992). Likewise, xylanase supplementation to diets based on wheat did not affect apparent ileal or total tract digestibility of CP or ileal digestibility of AA in weaner pigs (Diebold et al. 2005) and total tract digestibility of CP (N) in nursery and finishing pigs (Mavromichalis et al 2000). Supplementary xylanase in combination with β -glucanase had no influence on CP digestibility at any site of the digestive tract of weaned piglets fed diets based on main cereals and wheat bran (Högberg and Lindberg 2004). The same enzyme combination did not influence the total tract CP digestibility of growing or finishing pigs fed diets based on barley or rye, respectively (Thacker et al. 1992a), or of growing pigs fed diets containing barley, wheat and SBM with either low-mucilage or candle canola meal (Bell and Keith 1991). In addition, supplementing hull-less barley-based diets with xylanase did not affect apparent ileal or total tract digestibility of CP in young pigs, but improved apparent ileal digestibility of most of indispensable and dispensable AA (Yin et al. 2001).

As was the case for energy digestibility, plausible factors contributing to the inconsistency in AA and CP digestibility among studies might be the variations in substrate level of ingredients used, the level of supplementary xylanase added and the variations in genetic potential and age of experimental pigs. Collectively, the data indicate that effects of xylanase on protein and AA digestibility are dose dependent and may depend of the amount of arabinoxylans in the diet and whether the arabinoxylans are indeed a factor limiting protein and AA energy digestibility in the specific diet.

1.6.5.3 Effect on dry matter digestibility

Effects of xylanase supplementation on DM digestibility have been mixed, with some studies showing a favourable response. Addition of xylanase to diets based on wheat or its by-products improved apparent ileal and total-tract DM digestibility 4 and 1%, respectively, in grower pigs (Yin et al. 2000b). In weanling pigs, xylanase supplementation in combination with β -glucanase of diets containing hull-less barley improved both apparent ileal and total tract digestibility of DM 15 and 6%, respectively, (Baidoo et al. 1998) and of diets based on barley and pollard improved the apparent ileal digestibility of DM 5%, without affecting total tract digestibility (Graham et al. 1988). In addition, the same enzyme combination improved the total tract digestibility of DM in grower-finisher pigs fed diets containing normal or high fat oat (Thacker and Rossnagel 2005).

Xylanase supplementation did not affect DM digestibility in weanling pigs fed meal and pellet form rye-based diets (Thacker et al. 1991). Furthermore, addition of xylanase to wheat-based diets did not improve either ileal or total tract dry (organic) matter digestibility in weaner pigs (Diebold et al. 2005) or total tract digestibility of DM

in nursery and finishing pigs (Mavromichalis et al 2000). Xylanase, in combination with β -glucanase, in diets containing a variety of cereals, reduced dry (organic) matter digestibility 50% in the stomach of weaned piglets, without affecting the digestibility of organic matter at the other sites of the digestive tract (Högberg and Lindberg 2004). The same enzyme combination did not influence the apparent digestibility of DM in each of the four segments examined of the small intestine of weaned pigs fed wheat- and canola meal-based diets (Zijlstra et al. 2004). Moreover, supplementary xylanase together with β -glucanase did not influence total tract digestibility of DM in growing or finishing pigs fed diets based on barley or rye, respectively (Thacker et al. 1992a). Finally, supplementing hull-less barley based diets with xylanase did not affect apparent ileal or total tract digestibility of DM in young pigs (Yin et al. 2001). Combined, the data indicate that effects of xylanase on dry matter digestibility are dose dependent and may depend on the amount of arabinoxylans in the diet and whether the arabinoxylans are indeed a factor limiting dry matter digestibility in the specific diet.

Dry matter digestibility is generally not considered in least-cost diet formulation, but may have an importance in situations with a need to reduce dry matter excretion. Fresh or dry faeces output as a percentage of DM intake, or DM concentration in faeces of grower pigs fed diets based on wheat or wheat by-products supplemented with xylanase did not differ (Yin et al. 2000b).

1.6.5.4 Effect on performance

The consequences of xylanase supplementation on pig performance have been studied. Supplemental xylanase in corn- and wheat-based diets improved ADG 9.2%, ADFI 4%, and feed efficiency 5.3% in grower pigs (Van Lunen and Schulze 1996). With different

rates of xylanase supplementation, ADG and feed efficiency were improved linearly and quadratically in grower pigs fed wheat-based diets, achieving the highest responses with diets supplemented with 11,000 U g⁻¹ xylanase (Barrera et al. 2004). In another experiment, different rates of xylanase supplementation in combination with β -glucanase increased ADFI, ADG and body weight quadratically in weaned pigs fed diets based on wheat and canola meal, achieving the best performance with the diet supplemented with 2 g kg⁻¹ carbohydrase, resulting in 16, 13 and 7% higher ADFI, ADG and body weight, respectively, compared to the control diet (Zijlstra et al. 2004). However, enzyme supplementation reduced feed efficiency of pigs linearly. In addition, the same combination of supplementary enzymes improved ADG 5%, without affecting ADFI or feed efficiency, of growing pigs fed diets containing barley, wheat, SBM, and canola meal (Bell and Keith 1991).

In contrast, supplementing xylanase to rye-based diets in meal and pellet forms did not affect the ADG or ADFI in weanling pigs, but enzyme addition to the diet in meal form improved feed efficiency 10% (Thacker et al. 1991). Furthermore, xylanase supplementation did not improve performance of weanling pigs fed rye-based diets (Bedford et al. 1992). Similarly, xylanase supplementation did not improve performance of weaning pigs fed wheat- or barley-SBM diets (Inborr et al. 1993). Xylanase supplementation did not improve performance in nursery or finisher pigs fed wheat-based diets (Mavromichalis et al 2000). Supplementary xylanase in combination with β -glucanase did not influence the performance of growing or finishing pigs fed diets based on barley or rye, respectively (Thacker et al. 1992a), or of grower-finisher pigs fed diets containing normal or high fat oat (Thacker and Rossnagel 2005). Moreover, the same enzyme combination did not affect the feed intake or ADG of weaned piglets fed diets

composed of main cereals grains and wheat bran (Högberg and Lindberg 2004). Combined, the data indicate that effects of xylanase on growth performance are dose dependent and may depend of the amount of arabinoxylans in the diet and whether the arabinoxylans are indeed a factor limiting performance of pig fed the specific diet.

1.7 Summary

The value and use of distiller's feeds in swine rations are not fully realized due to the inconsistency of available information on its nutritional value for swine. Product variability is seemed to be a primary concern with the use of DDGS in swine rations. In addition, previous studies with corn DDGS revealed a reduced AA digestibility (Wahlstrom et al. 1970) and an inappropriate AA profile for pigs (Cromwell et al. 1983). The use of corn DDGS in swine rations has been tested with different inclusion rates in pigs of different growth stages. Information on the use of wheat DDGS in swine nutrition remains entirely lacking.

The relatively high fibre content in DDGS may have limited its use as an energy source in swine ration formulation. However, the DE dilution effect of high fibre content can be overcome with the counteracting effect of high fat content of DDGS, achieving a higher DE value compared to that of the original cereal grain (Newland and Mahan 1990). A great variability in DE and ME content of corn DDGS exists with samples from different sources (Spiehs et al. 2002) and different inclusion rates of the same sample (Spiehs et al. 2000). Different sources and inclusion rates of DDGS affect the energy digestibility differently causing variability in DE and ME contents.

The protein quality of DDGS is low due to deficiency of several indispensable AA (Newland and Mahan 1990). Particularly, the lack of lysine, the first limiting AA in swine nutrition, is critical. Some of the lysine is destroyed or made unavailable during the fermentation and/or drying processes of DDGS. A considerable variation in crude protein and AA composition exists in corn DDGS samples originated from various sources (Cromwell et al. 1993; Spiehs et al. 2002) and AA digestibility of DDGS in swine is lower than predominant cereal ingredients (Wahlstrom et al. 1970).

Despite the higher nutrient composition compared to the original cereal grain, the concentrated NSP fraction of DDGS may hamper the action of digestive enzymes in the small intestine and affect the digestion process of nutrients (van Barneveld et al. 1995, Grieshop et al. 2001). However, the hydrolysis of phytate-P during fermentation increased the availability of P in DDGS (Singsen et al. 1972 as referenced by Cromwell 1979).

Some studies reported a numerical reduction in VFI when corn DDGS is included in swine diets (Wahlstrom and Libal 1980; Whitney and Shurson 2004). Reasons for the reduction in VFI are still unclear but high fibre content, the AA profile that is not appropriate for pigs and high energy density can be considered as possible factors associated with reduced feed intake.

With the introduction of exogenous enzymes to alleviate the detrimental effects associated with NSP in feed ingredients, xylanase is widely used in commercial livestock rations to maximize the nutrient utilization from feedstuffs. Based on concentrated NSP components in wheat DDGS, use of supplementary xylanase would be worthwhile; however, the results of the studies conducted to evaluate the effect of xylanase supplementation of wheat-based diets on nutrient digestibility and growth

performance of pigs are controversial. Plausible factors contributing to the inconsistency in nutrient digestibility among studies might be the variations in substrate level of ingredients used, the level of supplementary xylanase added and the variations in genetic potential and age of experimental animals.

Based on the gathered information or lack thereof, the following research objectives were developed for this thesis:

- To determine the energy, AA and P digestibility of wheat and corn DDGS.
- To evaluate the effect of DDGS on DM, N and P excretion patterns.
- To determine whether feeding diets containing wheat or corn DDGS result in equal growth performance of grower-finisher pigs fed a wheat control diet.
- To investigate the influence of xylanase supplementation of wheat DDGS on ileal and total tract digestibility and digestible content of nutrients in grower-finisher pigs.
- To evaluate the effects of xylanase supplementation of wheat DDGS on DM, N and P excretion patterns.

2. NUTRITIONAL VALUE OF WHEAT AND CORN DISTILLER'S DRIED GRAIN WITH SOLUBLES: DIGESTIBILITY AND DIGESTIBLE CONTENTS OF ENERGY, AMINO ACIDS AND PHOSPHORUS, NUTRIENT EXCRETION AND GROWTH PERFORMANCE OF GROWER-FINISHER PIGS

2.1 Abstract

Digestibility and digestible contents of energy, amino acids (AA) and P in corn and wheat DDGS and wheat were determined together with N and P excretion and growth performance in grower-finisher pigs. In experiment 1, 12 barrows (64.6 ± 6.4 kg) were fitted with ileal T-cannulae and had restricted access ($2.6 \times$ maintenance) to a wheat control diet or one of three diets with 40% corn, wheat+corn (4:1) or wheat DDGS. For energy, apparent total tract digestibility was highest for wheat (85%; $P < 0.05$) and did not differ among DDGS (77 to 79%; $P > 0.10$). Total tract digestible energy (DE) was highest for corn DDGS ($4292 \text{ kcal kg}^{-1} \text{ DM}$; $P < 0.05$) and tended to differ among wheat+corn and wheat DDGS and wheat (4038, 4019, and 3807, respectively; $P = 0.06$). For lysine, apparent ileal digestibility (AID) was highest for wheat (71%; $P < 0.05$) and did not differ among DDGS (59 to 63%; $P > 0.10$). The apparent ileal digestible lysine content was highest for corn DDGS (0.51% DM; $P < 0.05$), intermediate for wheat+corn and wheat DDGS (0.45 and 0.42), and lowest for wheat (0.37%). For P, total tract digestibility was lowest for wheat (15%; $P < 0.05$) and did not differ among DDGS samples (53 to 56%; $P > 0.10$). Total N excretion was

highest for wheat+corn and wheat DDGS (55 and 58 g d⁻¹; $P < 0.05$), intermediate for corn DDGS (44) and lowest for wheat (36). Total P excretion did not differ among DDGS (11 g d⁻¹) and was lowest for wheat (8; $P < 0.05$). In experiment 2, 100 pigs (52.0 ± 3.3 kg) were fed a wheat-pea control diet or one of three 25%-DDGS (corn, wheat+corn or wheat) diets (3.375 Mcal DE kg⁻¹; 2.50 g AID lysine Mcal⁻¹ DE) for 5 wk. Overall, average daily feed intake (ADFI) and daily gain (ADG) were higher for wheat than DDGS ($P < 0.05$) but feed efficiency did not differ ($P > 0.10$). In summary, the nutritional value of wheat DDGS for swine is higher than wheat and lower than corn DDGS and feeding DDGS reduced growth performance, partly via a reduced ADFI, indicating that anti-nutritional factors in DDGS require further investigation.

2.2 Introduction

The DDGS is a by-product from the cereal grain-based ethanol and alcohol beverage industries (Newland and Mahan 1990). With the rapid growth of the ethanol industry, increasing quantities of DDGS are available for livestock rations, including wheat and corn DDGS in western Canada. In general, DDGS has higher concentrations of nutrients such as protein, fat, vitamins, minerals, and fibre than its parent grain. These chemical fractions are concentrated due to the removal of most of the cereal starch as ethanol and carbon dioxide during the fermentation process (Weigel et al. 1997).

Product variability and lack of information about the nutritional value of DDGS from different sources were primary reasons for the initial reluctance of using corn DDGS in swine diets. In addition, reduced AA digestibility (Wahlstrom et al. 1970), and an AA profile that is not well suited for pigs (Cromwell et al. 1983) were concerns. Due to the construction of new ethanol plants with modern fermentation and drying

technologies in the upper Midwest - USA, the nutritional value of corn DDGS for swine was re-addressed indicating that high quality corn DDGS with a less variable nutrient content has become available for the livestock industry (Shurson et al. 2000; Spiehs et al. 2002; Whitney and Shurson 2004); however, the digestible nutrient content of corn DDGS remains controversial. In contrast, the nutritional value of wheat DDGS for swine has never been described; therefore, its potential for the swine industry is not understood. The continuously increasing supply of corn and wheat DDGS suggests that evaluation of their nutritional value for swine is worthwhile to support development of cost-effective feeding programs. For any feed ingredient for swine, an understanding of the digestible nutrient content is critical to achieve precise diet formulation. In addition, nutrient excretion is a major concern for the swine industry due to its potential impact on the environment, and the effects of DDGS on nitrogen (N) and phosphorus (P) excretion patterns should thus be evaluated. Finally, the effects of DDGS on swine growth performance should be described, because the high fibre content in DDGS may reduce nutrient digestibility and voluntary feed intake, and thereby growth performance.

Based on currently available information on DDGS, it was hypothesized that wheat and corn DDGS can be included in swine diets as an alternative source of energy, AA and P, without affecting growth performance and nutrient excretion of grower-finisher pigs. The objectives of the present study were to determine the energy, AA and P digestibility of wheat and corn DDGS, to evaluate the effect of DDGS on DM, N and P excretion patterns, and to determine whether feeding diets containing wheat or corn DDGS result in equal growth performance of grower-finisher pigs fed a wheat control diet.

2.3 Materials and methods

2.3.1 Ingredients

The DDGS was obtained from two sources: corn DDGS from an unknown U.S. source, and wheat and wheat+corn DDGS from an ethanol plant (Mohawk Canada, Minnedosa, MB), using Hard Red Spring wheat as the feedstock. Wheat and corn are fermented occasionally together, and for production of the specific wheat+corn DDGS, the feedstocks wheat and corn were fermented in a 4:1 ratio. One specific sample of each DDGS was used for both studies.

2.3.2 Experimental Protocol

The animal protocols were approved by the University of Saskatchewan Committee of Animal Care and Supply, followed principles established by the Canadian Council on Animal Care (1993), and all experiments were performed at Prairie Swine Centre Inc. (Saskatoon, SK).

Digestibility Study. Four diets were tested in two experimental periods using cannulated grower-finisher pigs: one wheat control diet and three diets with DDGS. The sole source of energy and AA in the control diet was wheat whereas in the other three experimental diets, three different types of DDGS (corn, wheat+corn, or wheat) in addition to wheat were included as energy and AA sources (Table 2.1). Chromic oxide was included as an indigestible marker and diets were fortified to meet or exceed vitamins and mineral requirements [National Research Council (NRC) 1998]. A total of 12 barrows (64.6 ± 6.4 kg; PIC Canada Ltd., Airdrie, AB) surgically fitted with T-cannulae at the distal ileum were fed two experimental diets each in a controlled

Table 2.1 Composition of experimental diets used in the digestibility study

Ingredient (%)	Wheat control	Distiller's Dried Grains with Solubles		
		Corn	Wheat+corn	Wheat
Wheat	96.3	56.3	56.3	56.3
Distiller's dried grain with solubles				
Corn	-	40.0	-	-
Wheat+corn	-	-	40.0	-
Wheat	-	-	-	40.0
Limestone	1.1	1.1	1.1	1.1
Di-calcium phosphate	0.8	0.8	0.8	0.8
Mineral mix ^z	0.5	0.5	0.5	0.5
Vitamin mix ^y	0.5	0.5	0.5	0.5
Chromic oxide	0.4	0.4	0.4	0.4
Salt	0.4	0.4	0.4	0.4

^z Provided per kg of diet: Zn, 100 mg; Fe, 80 mg; Cu, 50 mg; Mn, 25 mg; I, 0.5 mg; Se, 100 µg.

^y Provided per kg of diet: vitamin A, 8,250 IU; vitamin D₃, 825 IU; vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; riboflavin, 5 mg; menadione, 4 mg; folic acid, 2 mg; thiamine, 1 mg; D-biotin, 200 µg; vitamin B₁₂, 25 µg.

change-over design (Li et al. 1994), for a total of 24 observations or six observations per diet.

Each 11-d experimental period consisted of a 6-d acclimation to experimental diets followed by a 3-d collection of faeces and urine and a 2-d collection of ileal digesta. The 12 pigs were housed in individual metabolism pens in one room. The dimensions of the pens were 1.5 (length) x 1.5 (width) x 0.9 m (height), and allowed freedom of movement of pigs during the entire experiment. The flooring of the pen was plastic-coated expanded metal and the siding was sturdy PVC-planking with a transparent plastic window to allow visual contact between pigs in adjacent cages. A single-space feeder and a bowl-drinker were located at the front of each pen. The room was maintained within thermo-neutral zone of the pigs, with a 14 hr light (0700 to 2100)/10 hr dark cycle. During the entire experiment, diets were provided in wet-mash form, in a 1:1 water to mash ratio. Daily feed allowance was adjusted to $2.6 \times$ maintenance [$2.6 \times 110 \text{ kcal DE kg}^{-1} \text{ body weight (BW)}^{0.75}$; NRC 1998] using an estimated diet DE content of $3,300 \text{ kcal kg}^{-1}$. Diets were fed in two equal meals at 0800 and 1600; 1.95 and 2.20 kg feed d^{-1} during the first and second period, respectively. Pigs had free access to water throughout the experiment.

During the collections, representative samples for each diet were collected. Faeces were collected for 3 d twice per day (0800 and 1600) using faeces collection systems (Van Kleef et al. 1994). Urine was quantitatively collected for 3 d during faeces collections for a minimum of two times per day using urine trays and collection bottles. Twenty mL of 12N HCl was added to the collection bottles prior to each collection to prevent volatilization of urinary N. Of the collected urine, 10% was sub-sampled. Digesta samples were collected for 2 d using bags containing 4% formic acid attached

to the opened cannula barrel for 10 hr. Collected faeces, urine and digesta samples were pooled per pig over collection period and stored with diet samples in a freezer maintained at -20°C .

Performance Study. Four diets (one wheat control diet and three DDGS diets) were tested in grower-finisher pigs (52.0 ± 3.3 kg; PIC Canada Ltd., Airdrie, AB). The main ingredients of the control diet were wheat, peas, and soybean meal. In the other three experimental diets 25% DDGS was included, replacing a portion of wheat, soybean meal and di-calcium phosphate. Diets were formulated, using the digestible nutrient content determined for each DDGS sample in the digestibility study, to meet or exceed the requirements for AA and other nutrients (NRC 1998), to $3.375 \text{ Mcal DE kg}^{-1}$ and $2.50 \text{ g apparent digestible lysine Mcal}^{-1} \text{ DE}$ (Table 2.2). A total of 100 grower pigs (40 barrows and 60 gilts) were selected based on BW and average daily gain (ADG) since birth, randomized within gender, and housed in one room, with five pigs per pen, in 20 pens. Barrows and gilts were penned separately and each of the four experimental diets was fed to two pens with barrows and three pens with gilts, for a total of 5 observations per diet. Two pigs from separate pens fed different diets were removed from the experiment due to tail biting and data collected from these pigs were excluded from statistical analyses.

The duration of the study period was 6 wk: a 1-wk adaptation to the room, pen, and pen mates followed by 5-wk feeding of experimental diets. The switch from grower diet to experimental diets was abrupt, i.e., without having a period of mixing the diets together. Pens were rectangular in shape measuring 2.36×1.68 m. The flooring of the pen was fully-slatted concrete and the siding was sturdy PVC-planking. A single-space

Table 2.2 Composition of experimental diets used in the performance study

Ingredient (%)	Wheat	Distiller's Dried Grains with Solubles		
	control	Corn	Wheat+corn	Wheat
Wheat	63.14	41.64	41.18	41.17
Peas	25.00	25.00	25.00	25.00
Distiller's dried grains with solubles				
Corn	-	25.00	-	-
Wheat+corn	-	-	25.00	-
Wheat	-	-	-	25.00
Soybean meal	7.20	4.40	4.40	4.40
Dicalcium phosphate	1.10	0.40	0.40	0.40
Canola oil	0.90	0.70	1.20	1.20
Limestone	0.75	1.00	1.00	1.00
Mineral mix ^z	0.50	0.50	0.50	0.50
Vitamin mix ^y	0.50	0.50	0.50	0.50
Sodium bicarbonate	0.29	0.29	0.29	0.29
L-Lysine-HCl	0.27	0.32	0.33	0.34
Salt	0.20	0.20	0.20	0.20
L-Threonine	0.10	0.05	-	-
DL-Methionine	0.05	-	-	-

^z Provided per kg of diet: Zn, 100 mg; Fe, 80 mg; Cu, 50 mg; Mn, 25 mg; I, 0.5 mg; Se, 100 µg.

^y Provided per kg of diet: vitamin A, 8,250 IU; vitamin D₃, 825 IU; vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; riboflavin, 5 mg; menadione, 4 mg; folic acid, 2 mg; thiamine, 1 mg; D-biotin, 200 µg; vitamin B₁₂, 25 µg.

feeder was located at the front and a nipple-drinker was located at the back of the pen. The room was maintained within thermo-neutral zone of the pigs, with a 14 hr light (0700 to 2100)/10 hr dark cycle. Diets were provided as a dry mash and diets and water were supplied ad libitum throughout the experiment.

Pigs were weighed at the initiation of feeding the experimental diets (d 0), and weekly thereafter, (d 7, 14, 21, 28, and 35). Feed disappearance was measured on each weigh day. The data were used to calculate ADG, ADFI, and feed efficiency (G:F).

2.3.3 Chemical Analyses

Faeces and digesta samples were thawed, homogenized, sub-sampled and freeze-dried. Urine was thawed, homogenized, sub-sampled and frozen. Ingredient, feed and freeze-dried faeces and digesta samples were ground through a 1-mm screen.

Chemical analyses were conducted in duplicate. For the digestibility study, the ingredient, feed, faeces and digesta samples were analysed for dry matter (DM) content by drying at 135 °C in an airflow type oven for 2 h (method 930.15; AOAC 1990). Feed, faeces and digesta samples were analysed for chromic oxide after ashing at 450 °C (Fenton and Fenton 1979) and gross energy using an IKA oxygen bomb calorimeter (model C 5003, IKA GmbH & Co. KG, Staufen, Germany). The calorimeter was operated in dynamic mode and benzoic acid was used as a caloric standard for calibration. Ingredient, feed and faeces samples were analysed for P according to the method described by Zasoski and Burau (1977) and urine was analysed for P using the molybdo-phosphate method (method 964.06; AOAC 1990). Feed, faeces and urine were analyzed for N by combustion (method 968.06; AOAC 1990) using a Leco

protein/N analyzer (model FP-528, Leco Co., St. Joseph, MI). Ingredient, feed and digesta samples were analysed for AA (method 982.30E; AOAC 1990) at the University of Missouri, Columbia, MO. In addition, the wheat sample used in the digestibility study and DDGS samples were analyzed for crude protein (CP; method 988.05; AOAC 1990), crude fat (method 954.02; AOAC 1990), ash (method 942.05; AOAC 1990), crude fibre (method 962.09; AOAC 1990), acid detergent fibre (ADF) and neutral detergent fibre (NDF) using an Ankom fiber analyzer (model ANKOM²⁰⁰, Ankom Technology, Fairport, NY) using sodium sulphite and α -amylase in the NDF procedure, NSP (Englyst and Hudson 1987), non-protein N (NPN; Licitra et al. 1996) and total phytate (Newkirk and Classen 1998).

Apparent ileal and total tract digestibility of nutrients for the wheat and DDGS was calculated using the indicator method (Adeola 2001). Standardized ileal digestibility of AA was calculated using the method of Yin et al. (2002), based on the mean endogenous AA composition of basal endogenous protein (Jansman et al. 2002).

2.3.4 Statistical analyses

Pig was considered the experimental unit for the digestibility study and pen for the performance study. Digestibility variables were analysed using the general linear models procedure of SAS (SAS Institute, Inc. 1996). Means were reported as least-square means [\pm pooled standard error of the mean (SEM)] and were separated using the probability of difference; $P < 0.05$ was considered significant to test the hypotheses. If pertinent, trends ($0.05 < P < 0.10$) were reported and $P > 0.10$ was considered non-significant. Performance variables were analyzed by analysis of variance using the

MIXED procedure of SAS using a statistical model with the following factors: diet, gender and diet x gender, and initial BW as a covariate. For the digestibility study, comparisons were made among ingredients and the wheat was compared to the three DDGS samples combined and the DDGS samples were compared with each other, using orthogonal contrasts whereas for the performance study, comparisons were made among diets and the control diet was compared to the three DDGS diets combined and the DDGS diets were compared with each other, using orthogonal contrasts.

2.4 Results

2.4.1 Chemical characteristics

The three DDGS samples contained more CP than wheat, and the CP content was highest for wheat DDGS (Table 2.3). The NPN content of wheat+corn and wheat DDGS was 6%-units higher than for corn DDGS. The crude fat content was 10%-units higher for corn DDGS than wheat DDGS. The ash, ADF, NDF, and crude fibre contents were similar among the DDGS samples, and were all higher than in wheat.

Similar to CP content, the total and indispensable AA contents were highest for wheat DDGS followed by wheat+corn and corn DDGS, with the exception of leucine and lysine (Table 2.3). The leucine and lysine content was highest for corn DDGS. The AA content in wheat DDGS doubled that of wheat, except for less of an increase for lysine.

The total P content was similar among DDGS samples and lowest in wheat (Table 2.4). Among DDGS samples, corn DDGS had the highest phytate content, but phytate was highest in wheat. In addition to phytate, wheat DDGS contained all lower inositol phosphate forms (IP2, IP3, IP4 and IP5) of phytate; corn and wheat+corn DDGS did

Table 2.3 Chemical characteristics of wheat, and corn, wheat+corn, and wheat distiller's dried grains with solubles (% DM)

Variable	Wheat	Distiller's Dried Grains with Solubles		
		Corn	Wheat+corn	Wheat
Moisture	11.8	11.8	8.0	8.1
Gross energy *	4490	5454	5257	5194
Crude protein	19.8	30.3	42.4	44.5
Non-protein nitrogen	4.6	5.4	12.4	10.2
Crude fat	1.8	12.8	4.7	2.9
Ash	2.1	4.8	5.0	5.3
Acid detergent fibre	2.7	14.6	19.5	21.1
Neutral detergent fibre	9.4	31.2	30.6	30.3
Crude fibre	2.4	7.0	7.8	7.6
<i>Amino acid</i>				
Arginine	0.91	1.33	1.64	1.77
Cysteine	0.48	0.70	0.89	0.96
Histidine	0.46	0.82	0.95	0.99
Isoleucine	0.68	1.14	1.50	1.59
Leucine	1.31	3.52	3.13	3.01
Lysine	0.52	0.83	0.72	0.72
Methionine	0.32	0.61	0.67	0.69
Phenylalanine	0.96	1.51	1.98	2.16
Threonine	0.54	1.09	1.22	1.28
Tryptophan	0.23	0.23	0.37	0.44
Valine	0.84	1.53	1.83	1.91
Total	19.48	28.32	37.25	40.21

* expressed in kcal kg⁻¹ DM

Table 2.4 Phytate and P profile of wheat, and corn, wheat+corn, and wheat distiller's dried grains with solubles (% DM)

Variable	Wheat	Distiller's Dried Grains with Solubles		
		Corn	Wheat+corn	Wheat
Phosphorus	0.40	0.86	1.02	1.10
Inositol diphosphate (IP2)	0.00	0.00	0.00	0.08
Inositol triphosphate (IP3)	0.00	0.09	0.09	0.09
Inositol quadruphosphate (IP4)	0.00	0.19	0.18	0.28
Inositol pentaphosphate (IP5)	0.00	0.45	0.33	0.64
Phytate (IP6)	1.39	0.92	0.62	0.81

not contain IP2. Detailed NSP analysis indicated that individual components such as arabinose and xylose were higher for DDGS samples than for the wheat (Table 2.5).

Overall for the measured characteristics, the content was lowest for the wheat sample among the four ingredient samples, except for phytate. Phytate content of wheat was 75% higher than for wheat DDGS, and wheat did not contain any of the lower forms of phytate.

2.4.2 Nutrient digestibility and content

The apparent ileal and total-tract digestibility of energy did not differ among DDGS samples ($P > 0.10$; Table 2.6); however, ileal and total-tract energy digestibility was 8 and 9%, respectively, higher for the wheat than for the DDGS samples ($P < 0.05$). The total-tract digestibility of P did not differ among the DDGS samples ($P > 0.10$), but was 30%-units higher for DDGS than the wheat ($P < 0.05$).

The AID of indispensable and total AA (Table 2.6) and the standardized ileal digestibility (SID) of the indispensable AA (Table 2.7) were similar among DDGS samples ($P > 0.10$), with the exception of the AID of phenylalanine and AID and SID of threonine. The AID of phenylalanine and threonine was similar for wheat+corn and wheat DDGS ($P > 0.10$), but lower for corn DDGS ($P < 0.05$) and the SID of threonine was lower for corn DDGS compared to wheat-based DDGS samples combined ($P < 0.05$). However, AID and SID of the indispensable and total AA were higher for wheat than DDGS ($P < 0.05$), except for the AID of arginine, leucine, methionine, threonine, tryptophan, and valine ($P > 0.10$).

Table 2.5 Non-starch polysaccharide profile of wheat, and corn, wheat+corn, and wheat distiller's dried grains with solubles (% DM)

Variable	Wheat	Distiller's Dried Grains with Solubles		
		Corn	Wheat+corn	Wheat
<i>Total NSP</i>				
Soluble	2.15	1.39	5.35	7.76
Insoluble	7.57	17.85	16.56	15.13
Total	9.72	19.24	21.91	22.89
<i>Xylose</i>				
Soluble	1.03	0.29	2.53	3.08
Insoluble	2.39	5.86	5.58	5.00
Total	3.42	6.15	8.11	8.08
<i>Arabinose</i>				
Soluble	0.68	0.21	1.22	1.58
Insoluble	1.64	4.06	3.51	3.29
Total	2.32	4.27	4.73	4.87
<i>Mannose</i>				
Soluble	0.04	0.29	0.43	0.34
Insoluble	0.10	0.66	0.59	0.58
Total	0.14	0.95	1.02	0.92
<i>Galactose</i>				
Soluble	0.23	0.07	0.27	0.51
Insoluble	0.40	1.37	1.04	0.82
Total	0.63	1.44	1.31	1.33
<i>Glucose</i>				
Soluble	0.07	0.46	0.73	1.09
Insoluble	3.03	5.79	5.78	5.38
Total	3.10	6.25	6.51	6.47

Table 2.6 Apparent ileal digestibility of energy and amino acids and total tract digestibility of energy and P in wheat, and corn, wheat+corn, and wheat distiller's dried grains with solubles

Variable ^z	Wheat	Distiller's Dried Grains with Solubles			Pooled
		Corn	Wheat+corn	Wheat	SEM
<i>Ileal digestibility (%)</i>					
Energy ^y	71.8	67.3	66.5	65.6	1.63
Amino acid					
Arginine	87.9	85.7	85.2	85.8	1.01
Cysteine ^y	84.1 ^a	77.4 ^b	78.2 ^b	77.0 ^b	1.22
Histidine ^y	87.6 ^a	81.5 ^b	80.8 ^b	80.9 ^b	1.08
Isoleucine ^y	83.5 ^a	78.0 ^b	79.4 ^b	79.0 ^b	1.07
Leucine	85.2	84.4	83.8	82.9	0.72
Lysine ^y	70.6 ^a	61.8 ^b	62.8 ^b	58.6 ^b	2.07
Methionine	85.0	84.2	84.2	82.6	0.91
Phenylalanine ^{yv}	87.9 ^a	84.0 ^b	86.2 ^{ab}	86.4 ^{ab}	0.77
Threonine ^v	73.7 ^a	69.4 ^b	74.4 ^a	72.8 ^a	1.04
Tryptophan	85.1	81.7	81.4	82.5	1.79
Valine	86.0	83.0	84.9	84.6	0.81
Total ^y	85.5 ^a	79.9 ^b	81.5 ^b	81.2 ^b	0.92
<i>Total tract digestibility (%)</i>					
Energy ^y	84.8 ^a	78.7 ^b	76.8 ^b	77.4 ^b	1.39
Phosphorus ^y	14.8 ^b	55.5 ^a	54.7 ^a	53.0 ^a	4.15

^zMeans are least-square means.

^y Wheat differs from the three DDGS ($P < 0.05$).

^v Corn DDGS differs from both wheat+corn and wheat DDGS ($P < 0.05$).

^{a-b} Within a row, means without a common letter differ ($P < 0.05$).

Table 2.7 Standardized ileal digestibility (%) of amino acids in wheat, and corn, wheat+corn, and wheat distiller's dried grains with solubles

Amino acid ^z	Wheat	Distiller's Dried Grains with Solubles			Pooled SEM
		Corn	Wheat+corn	Wheat	
Arginine ^y	92.2 ^a	88.6 ^b	87.6 ^b	88.0 ^b	1.01
Cysteine ^y	88.4 ^a	80.4 ^b	80.6 ^b	79.2 ^b	1.22
Histidine ^y	91.7 ^a	83.8 ^b	82.8 ^b	82.8 ^b	1.07
Isoleucine ^y	89.1 ^a	81.3 ^b	81.9 ^b	81.4 ^b	1.06
Leucine ^y	88.9 ^a	85.8 ^b	85.3 ^b	84.5 ^b	0.71
Lysine ^y	78.3 ^a	66.6 ^b	68.4 ^b	64.1 ^b	2.08
Methionine ^y	88.4 ^a	86.0 ^{ab}	85.9 ^{ab}	84.2 ^b	0.91
Phenylalanine ^y	91.4 ^a	86.2 ^b	88.0 ^b	88.0 ^b	0.77
Threonine ^{y^v}	85.0 ^a	75.0 ^c	79.4 ^b	77.5 ^{bc}	1.04
Tryptophan ^y	91.2	87.8	85.2	85.7	1.79
Valine ^y	92.4 ^a	86.5 ^b	87.8 ^b	87.4 ^b	0.81

^z Means are least-square means.

^y Wheat differs from the three DDGS ($P < 0.05$).

^v Corn DDGS differs from both wheat+corn and wheat DDGS ($P < 0.05$).

^{a-c} Within a row, means without a common letter differ ($P < 0.05$).

Among DDGS samples, the contents of apparent (Table 2.8) and standardized (Table 2.9) ileal digestible AA were highest for wheat DDGS followed by wheat+corn DDGS and corn DDGS ($P < 0.05$), except for leucine, lysine, methionine, and threonine. Specifically, ileal digestible leucine contents were lowest for wheat DDGS and highest for corn DDGS ($P < 0.05$). The ileal digestible lysine, methionine and threonine contents were similar for wheat and wheat+corn DDGS ($P > 0.10$); however, corn DDGS had the highest contents for lysine and the lowest contents for methionine and threonine ($P < 0.05$). Overall, ileal digestible contents of indispensable AA were lower for the wheat than DDGS ($P < 0.05$).

The apparent ileal and total tract DE contents were higher for corn DDGS than wheat+corn and wheat DDGS samples combined ($P < 0.05$), and the contents were lower for wheat than the DDGS samples combined ($P < 0.05$). Total tract DE content tended to be higher for wheat+corn and wheat DDGS than wheat ($P = 0.06$). The digestible P content was similar ($P > 0.10$) among DDGS samples and higher for DDGS than wheat ($P < 0.05$).

2.4.3 Nutrient excretion

The DM intake was similar among pigs fed wheat control and DDGS diets ($P > 0.10$). Pigs fed wheat+corn and wheat DDGS had a higher daily N and P intake than pigs fed corn DDGS ($P < 0.05$; Table 2.10). The N and P intake was lower for pigs fed wheat than pigs fed DDGS ($P < 0.05$).

The DM excretion on daily basis and as a percentage of intake was similar among pigs fed DDGS diets ($P > 0.10$; Table 2.10). The DM excretion was higher for pigs fed DDGS than the wheat control diet ($P < 0.05$).

Table 2.8 Apparent ileal digestible amino acid content (% DM), ileal and total tract digestible energy (kcal kg⁻¹ DM) and total tract digestible P (% DM) contents in wheat, and corn, wheat+corn, and wheat distiller's dried grains with solubles

Variable ^z	Wheat	Distiller’s Dried Grains with Solubles			Pooled
		Corn	Wheat+corn	Wheat	SEM
<i>Amino acid</i>					
Arginine ^{yvu}	0.80 ^d	1.14 ^c	1.40 ^b	1.52 ^a	0.02
Cysteine ^{yvu}	0.41 ^d	0.54 ^c	0.70 ^b	0.74 ^a	0.01
Histidine ^{yvu}	0.40 ^d	0.67 ^c	0.77 ^b	0.80 ^a	0.01
Isoleucine ^{yvu}	0.57 ^d	0.89 ^c	1.19 ^b	1.26 ^a	0.01
Leucine ^{yvu}	1.12 ^d	2.97 ^a	2.62 ^b	2.49 ^c	0.02
Lysine ^{yv}	0.37 ^c	0.51 ^a	0.45 ^b	0.42 ^b	0.02
Methionine ^{yv}	0.27 ^c	0.51 ^b	0.56 ^a	0.57 ^a	0.01
Phenylalanine ^{yvu}	0.84 ^d	1.27 ^c	1.71 ^b	1.87 ^a	0.01
Threonine ^{yv}	0.40 ^c	0.76 ^b	0.91 ^a	0.93 ^a	0.01
Tryptophan ^{yvu.}	0.20 ^c	0.19 ^c	0.30 ^b	0.36 ^a	0.01
Valine ^{yvu}	0.72 ^d	1.27 ^c	1.55 ^b	1.62 ^a	0.01
<i>Digestible energy</i>					
Ileal ^{yv}	3224 ^b	3671 ^a	3495 ^{ab}	3406 ^{ab}	82.1
Total tract ^{ywv}	3807 ^b	4292 ^a	4038 ^b	4019 ^b	73.4
Phosphorus ^y	0.06 ^b	0.48 ^a	0.56 ^a	0.59 ^a	0.04

^z Means are least-square means.

^y Wheat differs from the three DDGS ($P < 0.05$).

^w Wheat tended to differ from both wheat+corn and wheat DDGS ($P = 0.06$).

^v Corn DDGS differs from both wheat+corn and wheat DDGS ($P < 0.05$).

^u Wheat+corn DDGS differs from wheat DDGS ($P < 0.05$).

^{a-d} Within a row, means without a common letter differ ($P < 0.05$).

Table 2.9 Standardized ileal digestible amino acid content (% DM) in wheat and corn, wheat+corn, and wheat distiller's dried grains with solubles

Amino acid ^z	Wheat	Distiller's Dried Grains with Solubles			Pooled SEM
		Corn	Wheat+corn	Wheat	
Arginine ^{yvu}	0.84 ^d	1.18 ^c	1.44 ^b	1.56 ^a	0.02
Cysteine ^{yvu}	0.43 ^d	0.56 ^c	0.72 ^b	0.76 ^a	0.01
Histidine ^{yvu}	0.42 ^d	0.69 ^c	0.79 ^b	0.82 ^a	0.01
Isoleucine ^{yvu}	0.61 ^d	0.93 ^c	1.23 ^b	1.30 ^a	0.01
Leucine ^{yvu}	1.17 ^d	3.02 ^a	2.67 ^b	2.54 ^c	0.02
Lysine ^{yv}	0.41 ^c	0.55 ^a	0.49 ^b	0.46 ^b	0.02
Methionine ^{yv}	0.28 ^c	0.52 ^b	0.58 ^a	0.58 ^a	0.01
Phenylalanine ^{yvu}	0.88 ^d	1.30 ^c	1.74 ^b	1.90 ^a	0.01
Threonine ^{yv}	0.46 ^c	0.82 ^b	0.97 ^a	0.99 ^a	0.01
Tryptophan ^{yvu}	0.21 ^c	0.20 ^c	0.32 ^b	0.38 ^a	0.01
Valine ^{yvu}	0.78 ^d	1.33 ^c	1.61 ^b	1.67 ^a	0.01

^z Means are least-square means.

^y Wheat differs from the three DDGS ($P < 0.05$).

^v Corn DDGS differs from both wheat+corn and wheat DDGS ($P < 0.05$).

^u Wheat+corn DDGS differs from wheat DDGS ($P < 0.05$).

^{a-d} Within a row, means without a common letter differ ($P < 0.05$).

Table 2.10 Effect of distiller's dried grains with solubles on dry matter, N and P intake, excretion and retention (on DM basis)

Variable ^z	Wheat	Distiller's dried grains with solubles			Pooled
	control	Corn	Wheat+corn	Wheat	SEM
DM intake (g d ⁻¹)	1780	1838	1885	1796	35.2
DM excretion (g d ⁻¹) ^y	237 ^b	381 ^a	391 ^a	360 ^a	12.2
DM excretion (%) ^y	13.4 ^b	20.7 ^a	20.8 ^a	20.1 ^a	0.62
N intake (g d ⁻¹) ^{yv}	55.4 ^c	69.5 ^b	83.7 ^a	86.6 ^a	1.53
N excretion (g d ⁻¹)					
Faeces ^{yvu}	6.1 ^d	11.3 ^c	14.5 ^a	13.0 ^b	0.48
Urine ^{yv}	29.7 ^c	32.7 ^{bc}	40.7 ^{ab}	44.7 ^a	3.04
Total ^{yv}	35.7 ^b	44.1 ^b	55.2 ^a	57.7 ^a	2.94
N retention (g d ⁻¹) ^y	19.7	25.4	28.6	28.9	2.75
N retention (%)	35.6	36.4	33.9	33.2	3.69
P intake (g d ⁻¹) ^{yv}	11.4 ^c	15.2 ^b	16.6 ^a	16.6 ^a	0.29
P excretion (g d ⁻¹)					
Faeces ^y	6.9 ^b	8.5 ^a	8.4 ^a	8.4 ^a	0.29
Urine ^{yv}	1.1 ^b	2.0 ^a	2.7 ^a	2.7 ^a	0.20
Total ^y	8.0 ^b	10.5 ^a	11.0 ^a	11.1 ^a	0.33
P retention (g d ⁻¹) ^{yv}	3.4 ^b	4.6 ^a	5.6 ^a	5.6 ^a	0.27
P retention (%)	29.8	30.6	33.5	33.5	1.73

^z Means are least-square means.

^y Wheat differs from the three DDGS ($P < 0.05$).

^v Corn DDGS differs from both wheat+corn and wheat DDGS ($P < 0.05$).

^u Wheat+corn DDGS differs from wheat DDGS ($P < 0.05$).

^{a-d} Within a row, means without a common letter differ ($P < 0.05$).

Pigs fed wheat+corn DDGS excreted the highest amount of faecal N on a daily basis, followed by pigs fed the wheat DDGS and corn DDGS ($P < 0.05$; Table 2.10). Pigs fed DDGS had a higher faecal N excretion than pigs fed the wheat control ($P < 0.05$). Similarly, feeding DDGS increased urinary N excretion compared to the wheat control ($P < 0.05$) and urinary N excretion was lower for pigs fed diets with corn DDGS than for pigs fed diets with wheat-based DDGS in combined ($P < 0.05$). Total N excretion was higher for pigs fed wheat+corn and wheat DDGS than pigs fed corn DDGS ($P < 0.05$). Total N excretion did not differ between pigs fed the wheat control and corn DDGS ($P > 0.10$). Daily N retention was similar among pigs fed DDGS ($P > 0.10$), but was lower for pigs fed the wheat control than for pigs fed DDGS ($P < 0.05$).

Daily excretion of faecal, urinary and total P and P retention were lower for the pigs fed the control diet than for the pigs fed DDGS ($P < 0.05$). Urinary P excretion and P retention on daily basis were lower for pigs fed corn DDGS than for the pigs fed wheat-based DDGS combined ($P < 0.05$). Percentage of N and P retained did not differ among the four experimental diets ($P > 0.10$).

2.4.4 Growth performance

For the performance trial, final BW, ADG and ADFI did not differ among pigs fed diets containing DDGS for any of the weeks or the entire experimental period ($P > 0.10$; Table 2.11). Final BW, ADG and ADFI were higher for pigs fed the wheat control than for pigs fed DDGS ($P < 0.05$). However, DDGS did not affect feed efficiency of pigs ($P > 0.10$).

Table 2.11 Growth performance of pigs fed diets containing wheat, or corn, wheat+corn, and wheat distiller's dried grains with solubles

Variable ^z	Wheat	Distiller's Dried Grains with Solubles			Pooled
	control	Corn	Wheat+co	Wheat	SEM
<i>Body weight (kg)</i>					
d 0	51.70	51.88	53.39	51.17	0.44
d 7	60.19	59.58	59.04	59.45	0.44
d 14	67.01	65.99	65.83	65.78	0.44
d 21	74.33	72.30	72.22	72.33	0.44
d 28	81.31 ^a	78.89 ^{ab}	78.77 ^b	78.89 ^{ab}	0.44
d 35 ^y	88.06 ^a	85.82 ^{ab}	85.39 ^b	85.70 ^{ab}	0.44
<i>ADG (kg d⁻¹)</i>					
d 0 to 7	1.136	1.056	1.011	1.024	0.03
d 8 to 14	0.982	0.922	0.959	0.920	0.03
d 15 to 21	1.056	0.906	0.899	0.950	0.03
d 22 to 28	1.004	0.950	0.923	0.948	0.03
d 29 to 35	0.972	0.996	0.933	0.990	0.03
d 0 to 35 ^y	1.030 ^a	0.966 ^{ab}	0.945 ^b	0.967 ^{ab}	0.03
<i>ADFI (kg d⁻¹)</i>					
d 0 to 7	2.455	2.294	2.212	2.309	0.05
d 8 to 14	2.723	2.608	2.558	2.475	0.05
d 15 to 21	2.823	2.618	2.676	2.687	0.05
d 22 to 28	2.943 ^a	2.802 ^{ab}	2.664 ^b	2.863 ^{ab}	0.05
d 29 to 35	2.973	2.880	2.928	2.925	0.05
d 0 to 35 ^y	2.784 ^a	2.640 ^b	2.607 ^b	2.651 ^b	0.05
<i>Feed Efficiency</i>					
d 0 to 7	0.462	0.460	0.358	0.445	0.01
d 8 to 14	0.362	0.355	0.375	0.377	0.01
d 15 to 21	0.376	0.347	0.335	0.355	0.01
d 22 to 28	0.340	0.341	0.360	0.332	0.01
d 29 to 35	0.328	0.349	0.320	0.342	0.01
d 0 to 35	0.373	0.371	0.370	0.370	0.01

^z Means are least-square means.

^y Wheat differs from the three DDGS ($P < 0.05$).

^{a-b} Within a row, means without a common letter differ ($P < 0.05$).

2.5 Discussion

The chemical and nutritional properties of DDGS samples used in the present study varied considerably, as observed in previous studies (e.g., Carpenter 1970; Cromwell and Stahly 1986; Cromwell et al. 1993). Generally, the nutrient variability of DDGS cannot be entirely avoided, because DDGS is a by-product of a process primarily aimed at the production of ethanol. In the present study, the variation was large, because DDGS was produced from different feedstock and at two different ethanol plants. Overall, factors including the type of cereal grain used for fermentation, method of fermentation (batch or continuous), completeness or duration of the fermentation process, drying temperature and duration and the amount of dried solubles blended with distiller's dried grains can affect the chemical, physical and nutritional characteristics of DDGS (Carpenter 1970; Olentine 1986; Spiehs et al. 2002).

Generally, the characteristics of DDGS depend mainly upon the type and quality of the cereal grain used for ethanol production. The nutrient composition of DDGS reflects the nutrient content of original cereal grain, with a higher concentration of each remaining nutrient following starch removal (Sosulski and Tarasoff 1997; Weigel et al. 1997; Mustafa et al. 1999). Indeed, the content of the reported nutrients was higher for the DDGS samples than for the wheat sample used in the present study. The content of the NSP components were higher for wheat DDGS than for wheat, including glucose. Glucose is used by yeast for the production of ethanol during fermentation, but relatively higher amounts of glucose in DDGS samples indicate a less efficient utilization of glucose during the fermentation process.

One of the main observations of the present study was a lower total content of the first-limiting AA, lysine, in wheat-based DDGS compared to corn DDGS, despite a markedly higher CP and total AA contents in wheat DDGS. As a percentage of CP, the content of total lysine was 2.63, 2.74, 1.70 and 1.62% for wheat grain and corn, wheat+corn and wheat DDGS, respectively. This low total lysine content and high NPN content indicates that some of the lysine was damaged during the fermentation and/or drying processes of the wheat and wheat+corn DDGS. These two DDGS samples had a dark colour with slightly burnt odour, suggesting that the DDGS may have been overheated during the drying process. The comparatively low moisture content of wheat-based DDGS also indicated excessive drying. In contrast to wheat DDGS, the corn DDGS was lighter in colour with sweet and slightly fermented odour suggesting the lack of overheating during drying. A reduced total lysine content following overheating has also been described in soybean meal (Parsons et al. 1992). Following overheating, lysine forms complexes with carbonyl groups, the Maillard reaction, which is associated with a colour change. The Maillard reaction is stimulated by heat in the presence of moisture, the exact conditions present in the drying process (Patience et al. 1995). The total lysine data indicate that physical characteristics such as colour and odour should be considered to predict the nutritional value of DDGS, because these physical variables are related to nutrient digestibility and availability.

Despite the higher total nutrient content of DDGS relative to wheat, apparent ileal or total-tract digestibility of energy and AID or SID of indispensable AA were lower for DDGS compared to the wheat sample. Soluble and insoluble non-starch polysaccharides (NSP) are concentrated with other nutrients in DDGS, as a consequence of removal of most of the starch in the cereal grain during fermentation

(Mustafa et al. 1999). The increased level of NSP in DDGS, which was further confirmed via ADF and NDF analyses, likely contributed to the low digestibility of energy and AA in the present study. The NSP fractions may interfere with the action of digestive enzymes in the small intestine hampering the digestion process (Grieshop et al. 2001). The negative effects of NSP on DM, energy and AA digestibility have been well described (de Lange 2000; Jondreville et al. 2001). The inverse correlation between energy digestibility and NSP content (measured as NDF or ADF) has been reported for a variety of ingredients including barley (Fairbairn et al. 1999) and wheat (Zijlstra et al. 1999), a relationship that might be extended towards the low energy digestibility values observed for DDGS in the present study. Total endogenous AA losses increase with increased dietary content of NSP, especially ADF (Jondreville et al. 2001). Based on fibre content of DDGS, plausible factors contributing to low apparent AA digestibility in DDGS could be decreased enzymatic breakdown of dietary protein, thereby reduced absorption and increased undigested protein (AA) in digesta, increased endogenous AA losses (Jondreville et al. 2001) and binding of AA and peptides to fibre (Bergner et al. 1981, as referenced by Lenis et al. 1996).

Interestingly, P digestibility in DDGS was higher compared to wheat in the present study. Approximately 60 to 70% of the total P in a cereal-based diet is bound to phytate, and this phytate-P is not digested by swine (Baker, 1991). The undigested P is excreted in faeces, and may impact the environment negatively if not managed properly (Cromwell et al. 1993; NRC 1998). Wheat also contains intrinsic phytase that may support P digestibility in mash diets; however, wheat phytase is heat-labile (Jongbloed and Kemme 1990) and therefore destroyed during the fermentation and drying processes. Nevertheless, the effectiveness of intrinsic phytase of wheat is variable, and

was apparently low for the wheat sample in the present study based on the low P digestibility in wheat. The high P digestibility in DDGS was consistent with previous research (Singsen et al (1972) as referenced by Cromwell (1979) that indicated that P availability was enhanced in DDGS due to phytate-P hydrolysis by microbial phytase during fermentation. In the present study, the breakdown of phytate during fermentation was validated by the presence of less complex forms of phytate and the reduced phytate content in DDGS compared to the wheat sample. A higher P digestibility in DDGS compared to cereals suggests that feeding DDGS might improve P management in swine diets and manure, in contrast to N.

Digestible nutrient content is a reflection of total nutrient content and nutrient digestibility. Despite the highest energy digestibility in the wheat sample, apparent ileal and total-tract DE content remained lower in wheat than DDGS. The high fat content in DDGS, especially in corn DDGS, is associated with a higher gross energy content and therefore a higher DE content (Spiehs et al. 2002). The achieved DE content in DDGS indicates that the DE reduction caused by a high NSP content can be overcome with a high fat content. The substantially higher digestible P content in DDGS is due to higher P content and digestibility. Even though the ileal digestibility values of most of the indispensable AA were higher in the wheat sample than DDGS, apparent and standardized ileal digestible AA contents were higher for DDGS, especially for wheat-based DDGS, as a result of the higher AA content in DDGS samples. However, lower digestible content of lysine in wheat-based DDGS samples indicates heat damage of this AA during processing, reducing the digestibility.

In the present study, DM excretion was higher for pigs fed DDGS diets compared to pigs fed the wheat control diet, suggesting that feeding DDGS will increase manure volume. The increased DM excretion might be due to the high NSP content of DDGS, because addition of a fibre source to the diet elevates faecal DM excretion in pigs (Cherbut et al. 1988) and faecal DM excretion was related positively to the content of NSP, especially fermentable NSP in swine diets (Canh et al. 1998).

The higher N intake of pigs fed DDGS compared to wheat control diet was due to the higher CP content in DDGS, especially in wheat DDGS, and was directly related to an increased N excretion in faeces and urine. Almost 25% of the CP was NPN in wheat-based DDGS, suggesting that a large proportion of CP in wheat-based DDGS cannot be utilized by the pig and is partially responsible for the increased N excretion. The increased urinary N excretion indicates increased AA catabolism, suggesting an imbalanced AA profile or a limitation in one or more critical AA in DDGS, because excess AA are catabolized (Zervas and Zijlstra 2002). The increased AA catabolism might partly be attributed to the relatively low total and digestible lysine content of DDGS. About 50% of N excretion in pig manure can be attributed to the poor AA balance in the diet (de Lange, 2004). Interestingly, N retention as a percentage of N intake was not affected by DDGS in the diet, indicating that CP and AA contained in DDGS can be utilized by the pig.

The addition of DDGS to the diet increased N excretion in faeces and urine. As a percentage of total N excretion, faecal N excretion increased by 46% and urinary N excretion reduced by 10% compared to wheat, suggesting a direct relationship between N excretion pattern and DDGS inclusion in diets. Fermentable fibre will shift N excretion from urea in urine to bacterial protein in faeces (Lenis et al. 1996; Canh et al.

1998; Zervas and Zijlstra 2002). The bacterial N assimilation with the presence of fermentable NSP in the hindgut is the underlying reason for this phenomenon. Therefore, the NSP contained in DDGS might be fermentable as well. From the environmental point of view, faecal N is preferable to urinary N, because faecal N is volatilized slower than urinary N as ammonia (Canh et al. 1998).

Intake, excretion and retention of P were affected by DDGS. The higher P content in DDGS compared to wheat increased P intake of pigs fed DDGS diets, especially for wheat+corn and wheat DDGS. As a consequence of increased P intake with DDGS, increased amounts of P appeared in faeces and urine (Cromwell et al. 1993; Ekpe et al. 2002). The P excretion in swine mainly occurs in faeces. Both faecal and urinary P excretion was increased in pigs fed DDGS diets. The increased P digestibility in DDGS could not entirely overcome the increased content of digestible P in DDGS. The amount of digested P surpassed the P requirement of pigs fed DDGS in the present study, resulting in excess urinary P excretion (Ekpe et al. 2002). Despite the increased excretion, daily P retention was higher for pigs fed DDGS diets than pigs fed the wheat control diet. Similar to N retention, P retention as a percentage of P intake was not affected by dietary DDGS, indicating once more that P in DDGS was utilized to a large extent by pigs in the present study, and contributed directly to meeting the digestible P requirement (Jongbloed et al. 1999).

In the present study, inclusion of DDGS reduced growth performance of pigs without affecting feed efficiency. The DDGS contains a higher digestible energy and AA content than wheat, but following pre-characterization and incorporation of the digestible nutrient content information in diet formulation, DDGS caused a reduction in voluntary feed intake, ADG and final body weight. Reasons for the reduced feed intake

remain unclear; dietary inclusion of DDGS might negatively affect palatability (Whitney and Shurson 2004), or the high NSP content in DDGS might play a role. As for nutrients, unfavourable factors such as mold spores can be concentrated in DDGS, affecting the palatability and feed intake. Other possibilities for the reduced feed intake include an AA imbalance or an increased level of non-essential AA (Henry et al. 1992; Henry 1995 and Hahn et al. 1995) and high energy density due to relatively high fat content (Azain 2001) of DDGS. The increased level of NSP might cause increased fermentation in the large intestine of pigs (Grieshop et al. 2001). The increased fermentation might reduce feed intake through physically distending the colon, thereby delaying retention time (Cherbut et al. 1988).

The increased fermentation and increased CP content in the diet limiting in lysine is likely caused extra heat increment as a result of catabolism of excess AA (Henry et al. 1992). Indeed, feeding DDGS to swine increased N excretion, and thereby increased the requirement of energy for N excretion, leaving less energy available to support growth (Spiehs et al. 2002). In addition, the daily intake of digestible lysine was lower for pigs fed diets with DDGS than fed the wheat control diet, mainly due to a lower feed intake, because the diets were formulated to an equal digestible lysine content. The reduced digestible lysine intake for DDGS, together with reduced lysine availability in DDGS may have constrained protein accretion of pigs fed diets with DDGS, because lysine intake below requirement will suppress growth performance of grower-finisher pigs (Friesen et al. 1994).

The results of the present study indicate that wheat DDGS is a by-product with a nutritional value for swine that is higher than wheat. However, wheat DDGS has a lower nutritional value than corn DDGS, and the AA damaged by processing and high

NSP content might be major constraints to the nutritional value of wheat DDGS for pigs. Dietary DDGS might increase N and P excretion in pigs, but DDGS might shift a portion of N excretion from urine to faeces. Finally, the nutritional value of wheat DDGS can be enhanced by improving its digestible AA content with improved fermentation and drying processes and reducing the impact of NSP with supplementary enzymes.

3. EFFECT OF XYLANASE SUPPLEMENTATION OF WHEAT DISTILLER'S DRIED GRAINS WITH SOLUBLES ON ENERGY, AMINO ACID AND PHOSPHORUS DIGESTIBILITY AND DIGESTIBLE CONTENTS, AND NUTRIENT EXCRETION IN GROWER-FINISHER PIGS

3.1 Abstract

A study was performed to examine if xylanase supplementation of wheat DDGS would improve nutrient digestibility and reduce nutrient excretion in grower-finisher pigs. Wheat-based diets with or without 40% wheat DDGS were tested with or without supplementary xylanase (4,000 U kg⁻¹ feed) as a 2 x 2 factorial arrangement in a repeated Latin square design using eight cannulated barrows (29.4 ± 2.0 kg). Following a 6-day acclimation, faeces and urine were collected for 3 d, and ileal digesta for 2 d. The apparent ileal energy digestibility and DE content were not affected either by ingredient or xylanase ($P > 0.05$). The total tract energy digestibility and DE content were affected by ingredient ($P > 0.05$), but not by xylanase ($P > 0.05$). The total-tract energy digestibility was higher for wheat, but DE content was higher for wheat DDGS. The AID of arginine, isoleucine, leucine, phenylalanine, threonine and total AA, and SID of phenylalanine were higher ($P < 0.05$) for wheat DDGS compared to wheat. Xylanase improved AID and SID of most of the indispensable AA in wheat ($P < 0.05$), but not in wheat DDGS ($P > 0.05$). The ileal digestible AA contents were affected by ingredients ($P < 0.05$), but not by xylanase ($P > 0.05$). Digestible AA contents were higher for wheat DDGS than for wheat. The digestibility and digestible content of P

were affected by ingredient and xylanase ($P < 0.05$). The digestibility and digestible content of P were higher for wheat DDGS compared to wheat. Neither ingredient nor supplementary xylanase affected DM intake ($P > 0.05$). The DM excretion was affected by ingredient ($P < 0.05$), but not by xylanase ($P > 0.05$). Ingredients affected all N and P variables ($P < 0.05$), except percentage retained for both nutrients ($P > 0.05$). None of N variables ($P > 0.05$), but P intake and retention were affected by xylanase ($P < 0.05$). The DM excretion and, N and P intake, excretion and daily retention were higher for wheat DDGS than for wheat. Lack of beneficial response to supplementary xylanase might be due to inappropriate enzyme level or insufficient substrate level of wheat DDGS. In addition, unidentified factors associated with fermentation and drying processes might constrain the nutritional value of wheat DDGS. Further studies are required to determine the proper xylanase inclusion level and/or to identify the factors associated with reduced nutrient digestibility of wheat DDGS.

3.2 Introduction

Efficient feeding programs with maximum utilization of nutrients from feed ingredients are crucial to achieve high production efficiency in livestock industry, because feed remains the largest variable cost of swine production (Patience et al. 1995). The use of exogenous enzymes has been recognized as a common method of achieving this goal and enzymes help not only to improve nutrient utilization from feedstuffs, but also lessen the impact of swine production on the environment by reducing the manure output and pollution associated with manure (Classen 1998). The use of exogenous enzymes in livestock industry can be categorized into four categories: (1) removal of anti-nutritional factors (ANF); (2) increasing nutrient digestibility; (3) increasing NSP

digestibility; and (4) supplementing host endogenous enzymes (Classen 1998). However, the current use of exogenous enzymes in swine diets is primarily directed towards the potential degradation of NSP or phytic acid, to maximize nutrient utilization from feedstuffs.

In DDGS, the NSP (fibre) fraction of the cereal grain is concentrated with other nutrients, as a consequence of removal of most of the starch during fermentation (Mustafa et al. 1999). Similarly, the content of arabinoxylan, the predominant NSP component of wheat, escalates in wheat DDGS. The negative effect of dietary NSP on the nutrient digestibility is well-documented (Kennelly and Aherne 1980; Fairbairn et al. 1999; Zijlstra et al. 1999; de Lange 2000; de Lange et al. 2000; Jondreville et al. 2001; Yin et al. 2002). Therefore, supplementation with NSP-degrading enzymes [carbohydrases] has been recognized as one of the ways to alleviate negative effects associated with NSP, and xylanase is considered as the enzyme of choice for wheat-based diets due to its ability to degrade arabinoxylan. Accordingly, enhancement of the nutritional value of wheat DDGS may be achieved with xylanase supplementation. Experiments with swine however, often demonstrated inconsistent responses to xylanase supplementation. In some studies, xylanase supplementation improved nutrient digestibility (Rattay et al. 1998; Yin et al. 2000b; Barrera et al. 2004) or growth performance (Van Lunen and Schulze 1996; Barrera et al. 2004), while in other studies, xylanase supplementation failed to improve nutrient digestibility (Mavromichalis et al. 2000; Yin et al. 2001; Diebold et al. 2005) or growth performance (Bedford et al. 1992; Inborr et al. 1993; Mavromichalis et al. 2000). The prospective supply of increasing quantities of wheat DDGS in western Canada, suggests that improvement of its nutritional value for swine might be worthwhile (Chapter 2) to increase its potential use

in swine feeding programs. Moreover, possible ways to reduce nutrient excretion should be explored simultaneously, because effective nutrient management is a key to sustainable pork production (Honeyman 1996).

For the present study, it was hypothesized that the nutritional value of wheat DDGS can be enhanced using supplementary xylanase, improving nutrient digestibility and utilization in grower-finisher pigs. The objectives of the present study were to investigate the influence of xylanase supplementation of wheat DDGS on ileal and total tract digestibility and digestible content of nutrients in grower-finisher pigs. In addition, the effects of xylanase supplementation on DM, N and P excretion patterns were evaluated.

3.3 Materials and Methods

3.3.1 Ingredients

The wheat DDGS was obtained from an ethanol plant (Mohawk Canada, Minnedosa, MB), using Canada Western Prairie Spring wheat as a feedstock. A sample of feedstock wheat, which was fermented to generate the particular batch of DDGS, was obtained along with the DDGS to allow a direct comparison of the chemical characteristics of wheat DDGS with its parent grain.

3.3.2 Experimental Protocol

The animal protocols were approved by the University of Saskatchewan Committee of Animal Care and Supply, and followed principles established by the Canadian Council on Animal Care (1993).

Wheat-based diets with or without wheat DDGS were tested with or without supplemented xylanase as a 2 x 2 factorial experiment in a repeated Latin square design (Steel and Torrie, 1980). Wheat was considered the sole source of energy and AA in Wheat-based diets, whereas in the Wheat DDGS-diets, wheat DDGS was included by replacing 40% of wheat, as an energy and AA source (Table 3.1). Xylanase enzyme (Danisco Animal Nutrition, Marlborough, UK) was added to one each of Wheat-based and wheat DDGS diets; at a rate of 1 kg tonne⁻¹ of feed to reach an activity of 4000 units kg⁻¹ final feed. Chromic oxide was included as an indigestible marker and diets were fortified to meet or exceed vitamins and mineral requirements [National Research Council (NRC) 1998]. Information obtained from the previous digestibility study (Chapter 2) on energy, AA and P digestibility of wheat DDGS was taken into account during feed formulation. A total of eight barrows (29.4 ± 2.0 kg; PIC Canada Ltd., Airdrie, AB) surgically fitted with a T-cannulae at the distal ileum were each fed the four experimental diets in four experimental periods, for a total of 32 observations or eight observations per diet.

Each 11-d experimental period consisted of a 6-d acclimation to experimental diets followed by a 3-d collection of faeces and urine and a 2-d collection of ileal digesta. The metabolism room housed eight pigs in individual metabolism pens. The dimensions of the pens were 1.5 (length) x 1.5 (width) x 0.9 m (height), and allowed freedom of movement of pigs during the entire experiment. The flooring of the pen was plastic-coated expanded metal and the siding was sturdy PVC-planking with a transparent plastic window to allow visual contact between pigs in adjacent cages. A single-space feeder and a bowl-drinker were located at the front of each pen. The room was maintained within thermo-neutral zone of the pigs, with a 14 hr light (0700 to 2100)/10

Table 3.1 Composition of experimental diets

Ingredient (%)	Wheat		Wheat DDGS	
	Without xylanase	With xylanase	Without xylanase	With xylanase
Wheat	96.3	96.2	56.3	56.2
Wheat distiller's dried grain with solubles	0.0	0.0	40.0	40.0
Xylanase	0.0	0.1	0.0	0.1
Limestone	1.1	1.1	1.1	1.1
Di-calcium phosphate	0.8	0.8	0.8	0.8
Mineral mix ^z	0.5	0.5	0.5	0.5
Vitamin mix ^y	0.5	0.5	0.5	0.5
Chromic oxide	0.4	0.4	0.4	0.4
Salt	0.4	0.4	0.4	0.4

^z Provided per kg of diet: Zn, 100 mg; Fe, 80 mg; Cu, 50 mg; Mn, 25 mg; I, 0.5 mg; Se, 100 µg.

^y Provided per kg of diet: vitamin A, 8,250 IU; vitamin D₃, 825 IU; vitamin E, 40 IU; niacin, 35 mg; D-pantothenic acid, 15 mg; riboflavin, 5 mg; menadione, 4 mg; folic acid, 2 mg; thiamine, 1 mg; D-biotin, 200 µg; vitamin B₁₂, 25 µg.

hr dark cycle. During the entire experiment, diets were provided in wet-mash form, in a 1:1 water to mash ratio. Daily feed allowance was adjusted to 2.6 x maintenance ($2.6 \times 110 \text{ kcal DE kg}^{-1} \text{ BW}^{0.75}$; NRC 1998) using an estimated diet DE content of 3,300 kcal kg^{-1} . Diets were fed in two equal meals at 0800 and 1600; 1.08, 1.15, 1.22 and 1.32 kg feed d^{-1} during the first, second, third and fourth period, respectively. Pigs had free access to water throughout the experiment.

During the collections, representative samples for each diet were collected. Faeces were collected for 3 d twice per day (0800 and 1600) using faeces collection systems (Van Kleef et al. 1994). Urine was quantitatively collected for 3 d during faeces collections for a minimum of two times per day using urine trays and collection bottles. Twenty mL of 12 N HCl was added to the collection bottles prior to each collection to prevent volatilization of urinary N. Of the collected urine, 10% was sub-sampled. Digesta samples were collected for 2 d using bags containing 4% formic acid attached to the opened cannula barrel for 10 hr. Collected faeces, urine and digesta samples were pooled per pig over collection period and stored with diet samples in a freezer maintained at -20°C .

3.3.3 Chemical Analyses

Faeces and digesta samples were thawed, homogenized, sub-sampled and freeze-dried. Urine was thawed, homogenized, sub-sampled and frozen. Ingredient, feed and freeze-dried faeces and digesta samples were ground through a 1-mm screen.

Chemical analyses were conducted in duplicate. Ingredient, feed, faeces, and digesta samples were analysed for dry matter (DM) content by drying at 135 °C in an airflow type oven for 2 h (method 930.15; AOAC 1990). Feed, faeces and digesta samples were analysed for chromic oxide after ashing at 450 °C (Fenton and Fenton 1979) and gross energy (GE) using an IKA oxygen bomb calorimeter (model C 5003, IKA GmbH & Co. KG, Staufen, Germany). The calorimeter was operated in dynamic mode and benzoic acid was used as a caloric standard for calibration. Feed and faeces were analysed for P according to the method described by Zasoski and Burau (1977) and urine was analysed for P using the molybdo-phosphate method (method 964.06; AOAC 1990). Feed, faeces and urine were analyzed for N by combustion (method 968.06; AOAC 1990) using a Leco protein/N analyzer (model FP-528, Leco Co., St. Joseph, MI). Ingredient, feed and digesta samples were analysed for AA (method 982.30E; AOAC 1990) at the University of Missouri, Columbia, MO. In addition, wheat DDGS, wheat grain fermented to obtain the used batch of DDGS and wheat in the basal diet were analyzed for crude protein (CP; method 988.05; AOAC 1990), crude fat (method 954.02; AOAC 1990), ash (method 942.05; AOAC 1990), crude fibre (method 962.09; AOAC 1990), acid detergent fibre (ADF) and neutral detergent fibre (NDF) using an Ankom fiber analyzer (model ANKOM²⁰⁰, Ankom Technology, Fairport, NY) using sodium sulphite and α -amylase in the NDF procedure, NSP (Englyst and Hudson 1987), non-protein nitrogen (NPN; Licitra et al. 1996) and total phytate (Newkirk and Classen 1998).

Apparent ileal and total tract digestibility of nutrients was calculated using the indicator method (Adeola 2001). Standardized ileal digestibility of AA was calculated using the method of Yin et al. (2002), based on the mean endogenous AA composition of basal endogenous protein (Jansman et al. 2002). Using the digestible nutrient profile measured for wheat in the present study, the digestible nutrient profile for wheat DDGS was calculated, and reported as such.

3.3.4 Statistical analyses

The individual pig was considered the experimental unit. Variables were analyzed by analysis of variance as a 2 x 2 factorial arrangement, using the general linear models procedure of SAS (SAS Institute, Inc. 1996). The statistical model included effects for dietary treatment (ingredient, xylanase and two-way interaction terms). When there was an interaction effect, the simple effect was considered. For digestibility and nutrient excretion variables ingredients and diets were compared, respectively. Means were separated using the probability of difference ($P < 0.05$). Data are reported as least-square means.

3.4 Results

3.4.1 Chemical characteristics

The contents of CP, NPN, crude fat, ash, ADF, NDF and crude fibre were 22, 5, 3, 3, 13, 25 and 4%-units higher for wheat DDGS than for the wheat samples, respectively (Table 3.2). Similarly, the total and indispensable AA (Table 3.2) and the total P (Table 3.3) contents were higher for wheat DDGS compared to the wheat samples. In contrast, phytate content was 45% lower for DDGS than for the wheat samples. In addition to

Table 3.2 Chemical characteristics of wheat distiller's dried grains with solubles, wheat used for the wheat distiller's dried grains with solubles and wheat in the basal diet (% DM)

Variable	Wheat DDGS	Wheat used for DDGS	Wheat in the basal diet
Moisture	8.9	15.6	14.2
Gross energy *	5319	4478	4558
Crude protein	37.3	13.6	16.8
Non-protein nitrogen	8.2	3.0	3.8
Crude fat	5.0	1.8	1.9
Ash	5.0	1.8	2.0
Acid detergent fibre	16.7	3.7	4.7
Neutral detergent fibre	38.8	13.2	14.9
Crude fibre	7.0	2.5	3.0
<i>Amino acid</i>			
Arginine	1.49	0.59	0.81
Cysteine	0.64	0.28	0.32
Histidine	0.80	0.30	0.36
Isoleucine	1.41	0.45	0.57
Leucine	2.48	0.88	1.09
Lysine	0.81	0.40	0.56
Methionine	0.52	0.21	0.27
Phenylalanine	1.69	0.59	0.75
Threonine	1.05	0.37	0.48
Tryptophan	0.35	0.17	0.17
Valine	1.63	0.58	0.73
Total	31.28	12.24	15.40

* expressed in kcal kg⁻¹ DM

Table 3.3 Phytate and P profile of wheat distiller's dried grains with solubles, wheat used for the wheat distiller's dried grains with solubles and wheat in the basal diet (% DM)

Variable	Wheat DDGS	Wheat used for DDGS	Wheat in the basal diet
Phosphorus	0.99	0.34	0.40
Inositol diphosphate (IP2)	0.20	0.00	0.00
Inositol triphosphate (IP3)	0.30	0.00	0.00
Inositol quadruphosphate (IP4)	0.22	0.00	0.00
Inositolpentaphosphate (IP5)	0.35	0.00	0.00
Phytate (IP6)	0.65	1.15	1.08

phytate, wheat DDGS contained all lower inositol phosphate forms (IP2, IP3, IP4 and IP5) of phytate. Detailed NSP analysis indicated that individual components such as arabinose and xylose were higher for wheat DDGS than for the wheat samples, except glucose (Table 3.4).

3.4.2 Nutrient digestibility and content

The ingredients used in the present experiment affected the total-tract energy digestibility ($P < 0.05$), but not the apparent ileal energy digestibility ($P > 0.05$; Table 3.5). The total-tract digestibility of energy was 7% higher for wheat than for wheat DDGS. Neither apparent ileal nor total-tract energy digestibility was affected by supplementary xylanase or ingredient x xylanase interaction ($P > 0.10$).

The total-tract P digestibility was affected by ingredients and supplementary xylanase ($P < 0.05$), but not by ingredient x xylanase interaction ($P > 0.05$; Table 3.5). The total-tract P digestibility was 62% lower for wheat than for wheat DDGS and xylanase increased P digestibility 51%.

Ingredients used in the present experiment interacted significantly with supplementary xylanase for AID and SID of most of the indispensable AA ($P < 0.05$), except cysteine, lysine, methionine and tryptophan ($P > 0.05$; Table 3.6 and 3.7). Therefore, the individual treatment means and differences between the individual means were considered for those AA and shown in Figure 3.1. Specifically, the ingredients affected AID and SID of cysteine and lysine ($P < 0.05$). The AID of arginine, isoleucine, leucine, phenylalanine, threonine, and total AA and, SID of phenylalanine were higher for wheat DDGS compared to wheat ($P < 0.05$). The AID and SID of cysteine and lysine

Table 3.4 Non-starch polysaccharide profile of wheat distiller's dried grains with solubles, wheat used for the wheat distiller's dried grains with solubles and wheat in the basal diet (% DM)

NSP	Wheat DDGS	Wheat used for DDGS	Wheat in the basal diet
<i>Total NSP</i>			
Soluble	7.8	0.8	3.8
Insoluble	8.8	7.4	7.9
Total	16.6	8.2	11.7
<i>Xylose</i>			
Soluble	3.8	0.7	1.2
Insoluble	4.3	2.2	3.6
Total	8.1	2.9	4.8
<i>Arabinose</i>			
Soluble	2.1	0.4	0.8
Insoluble	2.8	1.5	2.2
Total	4.9	1.9	3.0
<i>Mannose</i>			
Soluble	0.41	0.06	0.08
Insoluble	0.26	0.05	0.14
Total	0.67	0.11	0.22
<i>Galactose</i>			
Soluble	0.53	0.20	0.30
Insoluble	0.35	0.27	0.28
Total	0.88	0.47	0.58
<i>Glucose</i>			
Soluble	1.1	0.0	1.4
Insoluble	1.1	3.3	1.6
Total	2.2	3.3	3.0

Table 3.5 Effect of ingredient (wheat and wheat distiller's dried grains with solubles) and xylanase supplementation on apparent ileal digestibility of energy and total tract digestibility of energy and phosphorus

Variable	Ingredient		Pooled SEM ^z	Xylanase		Pooled SEM ^z	P Value		
	Wheat	DDGS		-	+		Ingredient	Xylanase	Interaction
<i>Energy digestibility (%)</i>									
Apparent ileal	63.7	57.2	3.33	58.7	62.1	3.33	0.068	0.326	0.106
Total tract	78.9 ^a	73.4 ^b	2.05	76.3	76.0	2.05	0.015	0.871	0.951
<i>Phosphorus digestibility (%)</i>									
Total tract	18.8 ^b	49.4 ^a	3.74	27.2 ^b	41.0 ^a	3.74	<0.0001	0.001	0.085

^z Standard error of means, calculated from the error means square.

^{a,b} Within a row for each factor, means without a common letter differ ($P < 0.05$).

Table 3.6 Effect of ingredient (wheat and wheat distiller's dried grains with solubles) and xylanase supplementation on apparent ileal amino acid digestibility (%)

Amino acid	Ingredient		Pooled SEM ^z	Xylanase		Pooled SEM ^z	P Value		
	Wheat	DDGS		-	+		Ingredient	Xylanase	Interaction
Arginine	76.6	79.1	1.61	76.8	78.8	1.61	0.148	0.232	0.044
Cysteine	76.3 ^a	70.7 ^b	2.60	72.7	74.3	2.60	0.046	0.557	0.206
Histidine	76.0	75.4	1.71	74.4	77.1	1.71	0.735	0.121	0.021
Isoleucine	75.4	77.1	1.61	75.0	77.4	1.61	0.322	0.151	0.041
Leucine	77.2	79.4	1.55	77.1	79.5	1.55	0.168	0.143	0.022
Lysine	64.5 ^a	58.7 ^b	2.59	60.5	62.7	2.59	0.036	0.411	0.156
Methionine	79.1	78.3	1.53	77.7	79.6	1.53	0.620	0.223	0.129
Phenylalanine	79.2 ^b	82.8 ^a	1.39	79.8	82.2	1.39	0.018	0.103	0.015
Threonine	61.9	65.6	2.65	62.2	65.4	2.65	0.184	0.247	0.022
Tryptophan	78.6	80.2	2.10	78.6	80.2	2.10	0.462	0.460	0.104
Valine	73.2	72.8	1.88	71.7	74.3	1.88	0.803	0.187	0.042
Total	74.4	75.4	1.74	73.7	76.0	1.74	0.585	0.201	0.035

^z Standard error of means, calculated from the error means square.

^{a,b} Within a row, means without a common letter differ ($P < 0.05$).

Table 3.7 Effect of ingredient (wheat and wheat distiller's dried grains with solubles) and xylanase supplementation on standardized ileal amino acid digestibility (%)

Amino acid	Ingredient		Pooled SEM ^z	Xylanase		Pooled SEM ^z	P Value		
	Wheat	DDGS		-	+		Ingredient	Xylanase	Interaction
Arginine	81.4	81.7	1.61	80.6	82.6	1.61	0.880	0.235	0.044
Cysteine	82.9 ^a	74.0 ^b	2.60	77.7	79.2	2.60	0.003	0.562	0.207
Histidine	81.3	77.8	1.71	78.2	81.0	1.71	0.057	0.123	0.022
Isoleucine	82.1	79.8	1.61	79.7	82.1	1.61	0.175	0.148	0.041
Leucine	81.7	81.4	1.55	80.4	82.7	1.55	0.863	0.144	0.022
Lysine	71.7 ^a	63.6 ^b	2.59	66.5	68.7	2.59	0.005	0.413	0.158
Methionine	83.1	80.4	1.53	80.8	82.8	1.53	0.090	0.216	0.127
Phenylalanine	83.7	84.8	1.38	83.1	85.4	1.38	0.454	0.106	0.015
Threonine	74.6	71.4	2.65	71.4	74.6	2.65	0.246	0.247	0.022
Tryptophan	86.8	84.2	2.10	84.7	86.3	2.10	0.232	0.459	0.103
Valine	80.6 ^a	76.1 ^b	1.88	77.1	79.6	1.88	0.025	0.188	0.042

^z Standard error of means, calculated from the error means square.

^{a-c} Within a row, means without a common letter differ (P < 0.05).

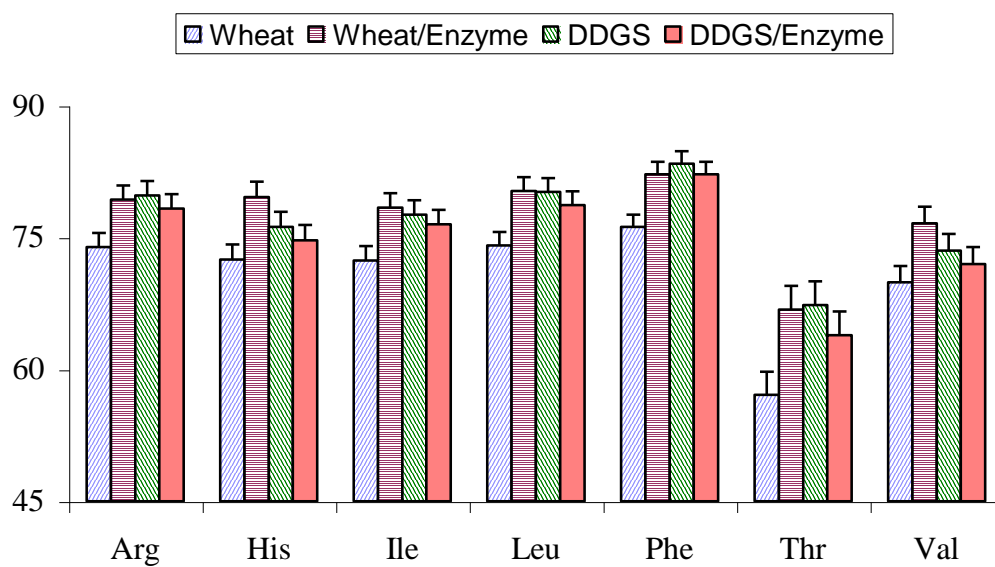
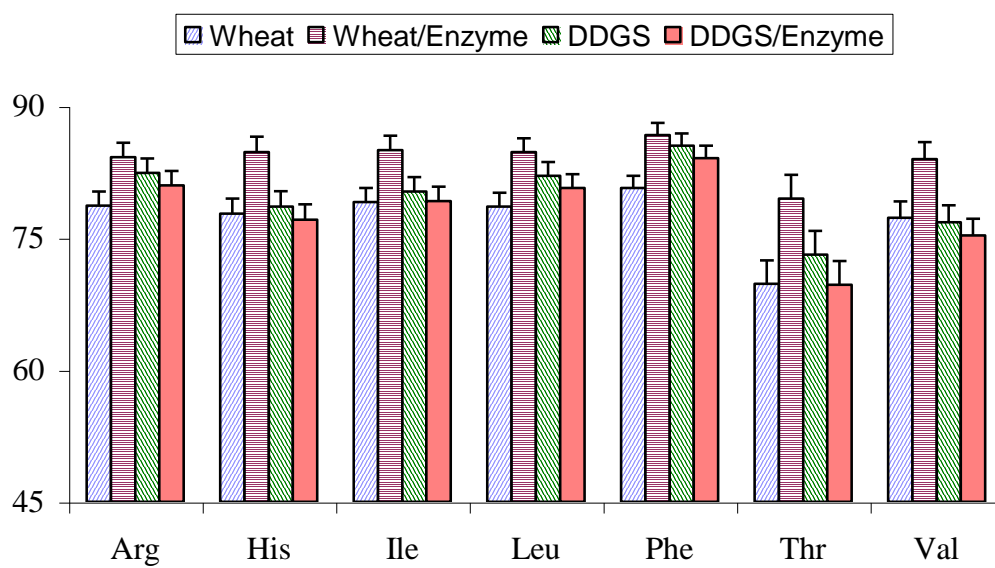
A**B**

Figure 3.1 Apparent (A) and standardized (B) ileal digestibility (%) of amino acids for individual dietary treatments.

were higher for wheat than for wheat DDGS ($P < 0.05$), while AID and SID of rest of the indispensable AA were similar for both ingredients ($P > 0.05$).

Overall, supplementary xylanase improved AID and SID of most of the indispensable AA in wheat ($P < 0.05$), with the exception of cysteine, lysine, methionine and tryptophan. The AID or SID of all indispensable AA were not affected with xylanase supplementation of wheat DDGS ($P > 0.05$).

Neither ingredients nor supplementary xylanase affected the apparent ileal DE content ($P > 0.05$; Table 3.8). The total-tract DE content was affected by ingredients ($P < 0.05$), but not by supplementary xylanase ($P > 0.05$). The total-tract DE content was higher for wheat DDGS than for wheat 7%. The ingredient x xylanase interaction did not affect either apparent ileal or total-tract DE content ($P > 0.05$). The total-tract digestible P content was affected by ingredients and xylanase ($P < 0.05$), but not by the ingredient x xylanase interaction ($P > 0.05$; Table 3.8). The digestible P content was 0.41%-units higher for wheat DDGS than wheat and xylanase improved digestible P content 32%.

Apparent and standardized ileal indispensable AA contents were affected by ingredients ($P < 0.05$), but not by supplementary xylanase or ingredient x xylanase interaction ($P > 0.05$; Table 3.8 and 3.9). Overall, apparent and standardized ileal digestible AA contents were higher for wheat DDGS than for wheat ($P < 0.05$).

3.4.3 Nutrient excretion

The DM intake of pigs was not affected by ingredients or xylanase ($P > 0.05$; Table 3.10). However, DM excretion on daily basis and as a percentage of intake was affected by ingredients ($P < 0.05$), but not by xylanase ($P > 0.05$). DM excretion on daily basis and as a percentage of intake was higher for wheat DDGS than for wheat 28 and 32%,

Table 3.8 Effect of ingredient (wheat and wheat distiller's dried grains with solubles) and xylanase supplementation on contents of apparent ileal digestible amino acid (% DM), ileal and total tract digestible energy (kcal kg⁻¹ DM) and total tract digestible phosphorus (% DM)

Variable	Ingredient		Pooled SEM ^z	Xylanase		Pooled SEM ^z	<i>P</i> Value		
	Wheat	DDGS		-	+		DDGS	Xylanase	Interaction
Arginine	0.62 ^b	1.18 ^a	0.02	0.89	0.91	0.02	<0.0001	0.491	0.090
Cysteine	0.24 ^b	0.45 ^a	0.02	0.35	0.35	0.02	<0.0001	0.871	0.428
Histidine	0.27 ^b	0.60 ^a	0.01	0.44	0.44	0.01	<0.0001	0.495	0.079
Isoleucine	0.43 ^b	1.09 ^a	0.02	0.76	0.77	0.02	<0.0001	0.584	0.184
Leucine	0.84 ^b	1.97 ^a	0.03	1.40	1.42	0.03	<0.0001	0.538	0.112
Lysine	0.36 ^b	0.48 ^a	0.02	0.41	0.43	0.02	<0.0001	0.578	0.266
Methionine	0.21 ^b	0.41 ^a	0.01	0.31	0.31	0.01	<0.0001	0.295	0.344
Phenylalanine	0.59 ^b	1.40 ^a	0.02	0.99	1.00	0.02	<0.0001	0.403	0.067
Threonine	0.30 ^b	0.69 ^a	0.02	0.49	0.50	0.02	<0.0001	0.722	0.081
Tryptophan	0.13 ^b	0.28 ^a	0.01	0.21	0.21	0.01	<0.0001	0.709	0.187
Valine	0.54 ^b	1.19 ^a	0.02	0.86	0.87	0.02	<0.0001	0.578	0.174
<i>Digestible energy</i>									
Ileal	2949	3044	168.9	2922	3072	168.9	0.587	0.387	0.131
Total tract	3654 ^b	3906 ^a	105.3	3789	3772	105.3	0.027	0.874	0.961
Phosphorus	0.08 ^b	0.49 ^a	0.04	0.25 ^b	0.33 ^a	0.04	<0.0001	0.043	0.788

^z Standard error of means, calculated from the error means square.

^{a,b} Within a row for ingredient, means without a common letter differ ($P < 0.05$)

Table 3.9 Effect of ingredient (wheat and wheat distiller's dried grains with solubles) and xylanase supplementation on standardized ileal digestible amino acid content (% DM)

Amino acid	Ingredient		Pooled SEM ^z	xylanase		Pooled SEM ^z	P Value		
	Wheat	DDGS		-	+		Ingredient	Xylanase	Interaction
Arginine	0.66 ^b	1.22 ^a	0.02	0.93	0.94	0.02	<0.0001	0.501	0.110
Cysteine	0.26 ^b	0.47 ^a	0.02	0.37	0.37	0.02	<0.0001	0.907	0.509
Histidine	0.29 ^b	0.62 ^a	0.01	0.45	0.46	0.01	<0.0001	0.422	0.118
Isoleucine	0.47 ^b	1.13 ^a	0.02	0.79	0.80	0.02	<0.0001	0.490	0.180
Leucine	0.89 ^b	2.02 ^a	0.03	1.45	1.46	0.03	<0.0001	0.552	0.110
Lysine	0.40 ^b	0.51 ^a	0.02	0.45	0.47	0.02	<0.0001	0.578	0.266
Methionine	0.22 ^b	0.42 ^a	0.01	0.32	0.32	0.01	<0.0001	0.289	0.460
Phenylalanine	0.63 ^b	1.44 ^a	0.02	1.02	1.04	0.02	<0.0001	0.439	0.097
Threonine	0.36 ^b	0.75 ^a	0.02	0.55	0.56	0.02	<0.0001	0.699	0.077
Tryptophan	0.15 ^b	0.30 ^a	0.01	0.22	0.22	0.01	<0.0001	0.921	0.294
Valine	0.59 ^b	1.24 ^a	0.02	0.91	0.92	0.02	<0.0001	0.575	0.163

^z Standard error of means, calculated from the error means square.

^{a,b} Within a row for ingredient, means without a common letter differ ($P < 0.05$).

Table 3.10 Effect of ingredient (wheat and wheat distiller's dried grains with solubles) and xylanase supplementation on dry matter, nitrogen and phosphorus intake, excretion and retention (on DM basis)

Variable	Ingredient		Pooled SEM ^z	Xylanase		Pooled SEM ^z	<i>P</i> Value		
	Wheat	DDGS		-	+		Ingredient	Xylanase	Interaction
DM intake (g d ⁻¹)	982.6	974.8	43.33	963.4	994.0	43.33	0.863	0.491	0.966
DM excretion (g d ⁻¹)	194.7 ^b	249.6 ^a	15.91	218.7	225.7	15.91	0.002	0.670	0.849
DM excretion (%)	19.4 ^b	25.6 ^a	1.15	22.4	22.6	1.15	<0.001	0.818	0.971
N intake (g d ⁻¹)	25.0 ^b	37.7 ^a	1.39	30.9	31.8	1.39	<0.0001	0.528	0.541
N excretion (g d ⁻¹)									
Faeces	5.0 ^b	8.5 ^a	0.55	6.8	6.8	0.55	<0.0001	0.938	0.398
Urine	9.1 ^b	14.7 ^a	1.31	12.1	11.7	1.31	0.0003	0.810	0.427
Total	14.1 ^b	23.2 ^a	1.62	18.8	18.5	1.62	<0.0001	0.865	0.352
N retention (g d ⁻¹)	10.9 ^b	14.5 ^a	1.33	12.1	13.3	1.33	0.015	0.392	0.619
N retention (%)	43.4	39.0	4.17	39.4	43.1	4.17	0.313	0.384	0.264
P intake (g d ⁻¹)	5.8 ^b	8.1 ^a	0.30	6.5 ^b	7.4 ^a	0.30	<0.0001	0.010	0.697
P excretion (g d ⁻¹)									
Faeces	3.5 ^b	4.6 ^a	0.22	3.9	4.2	0.22	0.0001	0.336	0.593
Urine	0.3 ^b	1.2 ^a	0.11	0.8	0.8	0.11	<0.0001	0.685	0.498
Total	3.9 ^b	5.8 ^a	0.25	4.7	4.9	0.25	<0.0001	0.472	0.434
P retention (g d ⁻¹)	1.9 ^b	2.4 ^a	0.20	1.8 ^b	2.5 ^a	0.20	0.027	0.003	0.136
P retention (%)	31.5	29.5	2.92	27.0 ^b	34.1 ^a	2.92	0.506	0.024	0.089

^z Standard error of means, calculated from the error means square.

^{a,b} Within a row for each factor, means without a common letter differ ($P < 0.05$).

respectively ($P < 0.05$). The ingredient x xylanase interaction did not affect DM variables ($P > 0.05$).

The ingredients affected all N and P variables ($P < 0.05$), except percentage retained for both nutrients ($P > 0.10$; Table 3.10). The inclusion of wheat DDGS in the diet increased N and P intakes 13 and 2 g d⁻¹, respectively ($P < 0.05$). Similarly, wheat DDGS in the diet increased N excretion in faeces, urine and the total N excretion 4, 6 and 9 g d⁻¹, and P excretion in faeces, urine and the total P excretion 1, 1 and 2 g d⁻¹, respectively ($P < 0.05$). Feeding of wheat DDGS increased N and P retention 4 and 1 g d⁻¹, respectively ($P < 0.05$).

None of N variables ($P > 0.05$), but P intake and, retention on daily basis and as a percentage of intake were improved by supplementary xylanase ($P < 0.05$; Table 3.10). Supplementary xylanase improved P intake and retention 1 and 0.7 g d⁻¹, respectively ($P < 0.05$). Similarly, xylanase increased P retention 7%-units, as a percentage of intake ($P < 0.05$). The N and P variables were not affected by an ingredient x xylanase interaction ($P > 0.05$).

3.5 Discussion

In the present study, the proximate fractions were similar for wheat samples in the DDGS and the basal diet, except CP, NPN and ADF, contents of which were 24, 27 and 27% higher, respectively, for the wheat in basal diet than for the wheat in DDGS. In addition, contents of most of the indispensable AA were higher for the wheat in basal diet than for the wheat in DDGS. Nutrient composition varies widely within the specific types of cereal grains, including wheat (Zijlstra et al. 1999). Nevertheless, similar to the previous study in Chapter 2, wheat DDGS contained higher concentrations of proximate

fractions and indispensable and total AA compared to wheat, except phytate, the content of which was higher for wheat than for wheat DDGS. The contents of the NSP components were higher for wheat DDGS than for wheat, except for glucose. Glucose is used by yeast for the production of ethanol during fermentation and as a result, contents of the other NSP components increase in wheat DDGS.

The removal of cereal starch during fermentation increases the concentration of the remaining nutrients as reported by Sosulski and Tarasoff (1997), Weigel et al. (1997) and Mustafa et al. (1999). Similarly, with the exception of phytate, the concentration of nutrients was higher for wheat DDGS used for the present study and reflected the nutrient composition of wheat used for fermentation. As observed in the previous study, wheat DDGS had a lower content of total phytate compared to wheat, due to breakdown of phytate-P during the fermentation process by microbial phytate (Singsen et al. 1972, as referenced by Cromwell 1979). In addition, yeast used for fermentation contributes a considerable portion of the nutrients present in DDGS (Ingeldew 1999). The contents of indispensable AA were relatively higher in the wheat DDGS used in Chapter 2, compared to the wheat DDGS used in the present study. Nevertheless, the content of total lysine was higher in the wheat DDGS used in the present study compared to the previous study, despite the lower CP, NPN and total AA contents in that DDGS sample. As a percentage of CP, the content of total lysine was 2.17%, compared to 1.62% in the wheat DDGS used in Chapter 2. This difference can be attributed to the variations associated with the drying process and the chemical characteristics of the parent wheat, and indicate that large variations exist in the digestible and available lysine content among DDGS samples. Indeed, the comparatively higher moisture content in the DDGS sample used in the present study indicated a lesser exposure of that sample to the heat,

compared to the previous DDGS sample, during the drying process, suggesting that less lysine might have been damaged during the drying process.

In the present study, the AID of energy was similar for wheat and wheat DDGS, but total-tract digestibility was higher for wheat than for wheat DDGS, similar to the previous study. In contrast to the previous study, AID and SID of indispensable AA were similar or higher for wheat DDGS compared to wheat, except cysteine and lysine. The AID and SID of cysteine and lysine were higher for wheat than for wheat DDGS. Dietary NSP hinders the digestion process in the small intestine interfering with the action of digestive enzymes (van Barneveld et al. 1995; Grieshop et al. 2001). Therefore, the concentrated NSP in wheat DDGS may affect the digestibility of its nutrients. However, the unaffected or higher AID and SID of indispensable AA in wheat DDGS indicate that the lower NSP content of wheat DDGS used in the present study did not negatively affect the AA digestibility. Similarly, endogenous AA losses are positively correlated to dietary NSP content (Jondreville et al. 2001); thus, the lower NSP content of the wheat DDGS used in the present study appears to be a major reason for the improved or unaffected digestibility values for AA.

Similar to the previous study, the wheat DDGS sample used in the present study contained less complex forms of phytate and the total phytate content was lower compared to wheat due to the breakdown of phytate by microbial phytase during fermentation. The relatively high total content and digestibility of P in wheat DDGS compared to cereals suggest that environmental and economical benefits of DDGS for the management of P in swine diets and manure may exist, because P is the third most expensive nutrient in the diet and if not managed properly, may have a major environmental impact (Cromwell et al. 1993; Liu et al 1998; NRC 1998).

In the present study, neither apparent ileal nor total tract digestibility of energy were affected by supplementary xylanase and AID and SID of most of the indispensable AA were improved by xylanase supplementation of wheat, but not of wheat DDGS. Pigs have demonstrated inconsistent responses to xylanase supplementation among experiments. Findings of the present study are in agreement with previous observations on inconsistency in nutrient digestibility upon xylanase supplementation of swine diets. Supplementary xylanase (650 U g^{-1}) in diets with approximately 80% rye did not improve the total-tract energy digestibility in weanling pigs (Thacker et al. 1991). Similarly, supplementing xylanase to diets with 77% hull-less barley (750 U kg^{-1}) or 60% wheat (5600 EXU kg^{-1}) did not improve either apparent ileal or total tract energy digestibility in young or weaner pigs, respectively (Yin et al. 2001b; Diebold et al. 2005). In addition, supplementary xylanase (5600 EXU kg^{-1}) in wheat -based diets did not affect the apparent ileal AA digestibility (Diebold et al. 2005). In contrast, xylanase supplementation (5000 U kg^{-1}) of diets based on wheat or its by-products (76%) improved apparent ileal AA digestibility and, apparent ileal and total-tract energy digestibility in grower pigs (Yin et al. 2000b) Similarly, with the increase in the rate of xylanase supplementation, apparent ileal AA digestibility improved linearly and quadratically in grower pigs fed diets with 98% wheat, achieving the highest digestibility values in diets supplemented with $11,000 \text{ U g}^{-1}$ enzyme activity (Barrera et al. 2004).

Reasons for the lack of response in nutrient digestibility to supplementary xylanase are still unclear. Probably, the level of xylanase included in wheat DDGS diet might not be appropriate to show a significant response, because the content of arabinose and xylose in wheat DDGS used in the present study was higher compared to the normal

range (2.6 – 4.1 and 4.3 – 6.5% DM, respectively) existing in wheat (Zijlstra et al. 1999). On the other hand, arabinoxylans might not be a factor limiting nutrient digestibility in the experimental diets used in the present study. Ferulic acid that exists in plant cell wall may form cross-linkages between lignin and carbohydrates or between carbohydrates by ester bonds (Eraso and Hartley 1990; Iiyama et al. 1994; Bartolomé et al. 1997). These cross-linkages may inhibit the action of supplementary xylanase. In addition, the feedstock used for ethanol production is exposed to a number of physical as well as chemical activities during fermentation and drying processes. These procedures may change the nature of NSP, including arabinoxylans, thereby preventing xylanase from being effective. Furthermore, the particle size of wheat and wheat DDGS might not be similar, because wheat DDGS was included in the experimental diets without further grinding. Therefore, the particle size of wheat DDGS might not be appropriate for the optimum efficacy of xylanase, because dietary particle size is a critical factor that should be considered before carbohydrase application (Oryschak et al. 2002). Finally, unidentified factors associated with wheat DDGS might also be a constraint to the effectiveness of xylanase. Interestingly, xylanase supplementation affected the digestibility of P, improving the total-tract P digestibility.

Similar to the previous study, the apparent ileal DE content was similar for both wheat and wheat DDGS in the present study, but in contrast to the previous study, the total-tract DE content was higher for wheat DDGS than for wheat. The fat content is higher for wheat DDGS than for wheat and therefore the DE dilution effect by the high NSP content in wheat DDGS could be overcome, as Spiehs et al. (2002) reported for corn DDGS. Similar to the previous study, the apparent and standardized ileal digestible AA contents were significantly higher for wheat DDGS than for wheat and this can be

attributed to the higher AA content in wheat DDGS. The digestible P content was 0.41%-unit higher for wheat DDGS, similar to 0.53%-unit increase in the previous study, compared to wheat. The significantly higher available P content in wheat DDGS is apparently due to higher content as well as availability of P in wheat DDGS.

As for the energy digestibility, supplementary xylanase did not affect either apparent ileal or total-tract DE content, since digestible content is partly a reflection of digestibility. Similarly, contents of apparent ileal or standardized AA were not affected by xylanase supplementation. Similar to the total-tract P digestibility, the digestible P content was improved by supplementary xylanase, demonstrating the correlation between nutrient digestibility and digestible nutrient content.

Similar to the previous study, DM excretion was higher for pigs fed wheat DDGS compared to pigs fed wheat, indicating an increased faecal output with the inclusion of wheat DDGS to the diet. Inclusion of a fibre source to the diet, fermentable fibre in particular, increases faecal DM excretion in pigs (Cherbut et al. 1988; Canh et al. 1998). Therefore, the increased DM excretion can be attributable to the high NSP content of wheat DDGS. On the other hand, supplementing the diets with xylanase had no additional benefits in reducing manure output. This was mainly due to the lack of response of nutrient digestibility to supplementary xylanase, because highly digestible ingredients in swine diets reduce faecal output (Zhang et al. 2003), and swine manure volume can be reduced with feed ingredients, which are better utilized by pigs (Grandhi 2001).

The inclusion of wheat DDGS into the diet increased N intake, faecal and urinary excretion and retention on daily basis compared to strictly feeding wheat, but not as a percentage of intake, similar to the previous study. The increase can be attributed to the

higher CP content in wheat DDGS. The increased urinary N excretion indicates an elevated AA catabolism and N excretion, despite the increase in digestible contents of all indispensable AA and N retention. The poor AA balance in the diet would be responsible for about 50% of N excretion in pig manure (de Lange 2004). Thus, the increase suggests that the additional digested and absorbed N was not retained due to an imbalanced AA profile or a limitation in one or more critical AA in wheat DDGS, because the excess AA are catabolized, resulting an increase in urinary N excretion. Specifically, lysine is limiting in western Canadian feed grains, including wheat (Patience et al. 1995). This together with other factors like Maillard reaction makes the content of lysine low and unavailable in wheat DDGS. The increased AA catabolism might therefore partly be attributed to the relatively low total and digestible contents of the first limiting AA, lysine, in wheat DDGS. In accordance with the results of the previous study, N retention as a percentage of intake was not affected by wheat DDGS in the present study, indicating a direct correlation between N intake and retention at levels provided in the experimental diets.

The inclusion of wheat DDGS in the diet elevated N excretion mainly in the faeces; faecal N excretion was increased 3% and urinary N excretion was reduced 2%, as a percentage of total N excretion, with the addition of wheat DDGS to the diet. These results are in agreement with the previous study that indicated a direct positive correlation between the N excretion pattern and the presence of DDGS in the diet, supported by some previous studies which observed enhanced faecal N excretion and depressed urinary N excretion, with the addition of more fermentable fibre to the diet (Morgan and Whittemore 1988; Mroz et al. 1993; Lenis et al. 1996; Canh et al. 1997; Canh et al. 1998; Zervas and Zijlstra 2002). Fermentable NSP in diet and excess N in

circulation serve as energy and N sources, respectively, for bacterial protein synthesis in the hindgut of pig. Therefore, the fermentable portion of NSP in wheat DDGS may shift an amount of N excretion from urine to faeces. In addition, reduced AA digestibility despite the high CP and total AA contents of wheat DDGS and, increased NPN content of wheat DDGS can be considered as the other factors associated with the N excretion pattern. From the environmental point of view, faecal N is preferable to urinary N, because faecal N (mainly bacterial protein) is less susceptible to rapid decomposition than urinary N (mainly urea), which is easily converted into ammonium and CO₂ by faecal urease enzyme (Canh et al. 1998; Mroz et al. 2000).

The unaffected N intake by supplementary xylanase in the present experiment suggested that N intake was not facilitated by enzyme supplementation. Accordingly, N excretion and retention patterns were not affected as well.

Feeding diets with wheat DDGS to pigs increased P intake, faecal and urinary excretion and retention on daily basis compared to strictly feeding wheat, but not as a percentage of intake, similar to the previous study. The relatively higher P content in wheat DDGS increased the P intake of pigs fed the diets with wheat DDGS, as observed in the previous study. As a result of increased P intake with wheat DDGS, an elevated amount of P was excreted in faeces and urine, indicating the positive correlation between intake and excretion of P, reported by previous studies (Cromwell et al. 1993; Jongbloed et al. 1993; Liu et al. 1998; Ekpe et al. 2002). The increased level of digestible P in wheat DDGS diets was beyond the expected increase in P digestion and absorption, as reported by Ekpe et al. (2002) and resulted an elevated P excretion. Despite the increased excretion, P retention on daily basis was improved with the inclusion of wheat DDGS into the diet. Similar to N retention, P retention as a

percentage of intake was not affected due to the presence of DDGS in the diet. This observation reveals the direct correlation between P intake and retention at the levels provided in the experimental diets. This can be considered as an important observation, because the main determinant of available/digestible P requirement is P retention in the animal's body, while P losses with urine and the integument contribute little to P requirements (ARC 1981; Hendriks and Moughan 1993; Jongbloed et al. 1999).

Unlike for N, supplementary xylanase improved P intake and retention, without affecting excretion. Xylanase supplementation increased P intake 14%, indicating that P intake was facilitated by supplementary xylanase. The P excretion was not affected with the increased intake, because the level increased with the support of xylanase might be within the limit that can be digested and absorbed, and P retention was increased to meet the requirement accordingly. Hence, all these observations, together with increased P digestibility by supplementary xylanase suggests that pigs fed wheat control diets were still not be able to meet their P requirement or just met their P requirement.

The results of the present study indicate that the recommended inclusion rate of supplementary xylanase is not appropriate to improve energy and AA digestibility of wheat DDGS. However, P intake and digestibility are improved by the level of xylanase used in the present study, improving P retention. No additional benefits on faecal volume and nutrient excretion can be expected with the recommended level of supplementary xylanase in wheat DDGS. Overall, nutrient digestibility and utilization of wheat DDGS cannot be improved with the level of xylanase used in the present study and further studies are required to evaluate the proper xylanase inclusion level and/or to identify the factors associated with reduced nutrient digestibility of wheat DDGS.

4. GENERAL DISCUSSION

Two studies described in two chapters were conducted with DDGS in grower-finisher pigs. In the general discussion, the combined research is discussed, including limitations in the present research, implications and future research.

4.1 Nutritional characteristics of distiller's dried grains with solubles

The chemical and nutritional properties varied among the four DDGS samples and between the two wheat DDGS samples used in both studies, indicating the variation in nutrient profile among DDGS samples. Overall, the digestible nutrient content was higher for the DDGS samples than for the wheat samples. Based on the described relationship between physical characteristics and nutrient composition of DDGS samples, physical characteristics such as colour, odour and moisture content should be considered to predict the nutritional value of DDGS.

Variability exists in nutrient digestibility among DDGS samples as well. In Chapter 2, digestibility of energy and indispensable AA were lower for DDGS than wheat, while in Chapter 3, the AID of energy and AID and SID of indispensable AA were similar or higher for wheat DDGS than wheat. This difference might be attributed to NSP content of DDGS and the lower NSP content of wheat DDGS used in Chapter 3 resulted in a higher nutrient digestibility than the wheat DDGS used in Chapter 2. Phosphorus digestibility was higher in DDGS than wheat in both studies, and coincided with a partial breakdown of phytate-P during fermentation. Overall, the higher digestible nutrient contents for DDGS than wheat in both chapters resulted thus foremost from a higher total nutrient content in DDGS samples.

The feeding of DDGS to swine will increase DM and N excretion by pigs. Dietary addition of DDGS increased faecal DM excretion in Chapter 2. The higher CP and NPN contents in DDGS compared to wheat indicate that less of dietary N in DDGS can be utilized by pigs, a main factor responsible for higher N excretion. Using diets formulated properly for digestible P content, P excretion of pigs fed DDGS is expected to be reduced, because less inorganic P needs to be supplemented to meet requirements.

The reduced VFI associated with DDGS reduced growth performance of pigs, without affecting feed efficiency. A high NSP content, an improperly-balanced AA profile or a high fat content can be considered as primary reasons for the reduced feed intake. In summary, DDGS contains nutrients valuable to swine, but nutrient excretion might be increased and growth performance reduced in pigs fed DDGS.

4.2 Effect of xylanase supplementation on nutritional characteristics of wheat distiller's dried grains with solubles

Xylanase supplementation of wheat DDGS did not improve nutrient digestibility or digestible nutrient content in Chapter 3, similar to previous studies without beneficial effects of supplementation on nutrient digestibility. An inappropriate substrate to-enzyme ratio due to high amount of arabinoxylans in wheat DDGS might be a main factor for the lack of response to supplementary xylanase. In addition, improper particle size, concentrated ferulic acid or unidentified factors associated with fermentation, drying and processing might have limited the efficacy of supplementary xylanase.

4.3 Limitations to the present studies

Several limitations might have contributed to the observed results. Previous studies with corn DDGS suggest that nutrient digestibility and growth performance of pigs primarily depend on the production stage of pigs and inclusion rate of DDGS. For the growth performance study in Chapter 2, diets could have been formulated with a range of DDGS inclusion rates and tested with pigs of different age groups. The expanded experimental design would however have increased the number of treatments and experimental pigs, making the study much larger and logistically more difficult to manage.

In Chapter 3, ingredients were not analyzed for NSP prior to diet formulation so that the level of substrate (arabinoxylans) was unknown. The supplementary xylanase was included at the level recommended by the manufacturer, and therefore likely not matched with substrate level. Subsequently, the analyzed NSP profile of wheat DDGS revealed that levels of arabinose and xylose were much higher compared to the normal range existing in wheat and the used xylanase level did not improve nutrient digestibility. As reported previously, the response to xylanase supplementation might depend on level of supplementation. The precise inclusion rate of xylanase could have been determined with a range of inclusion levels, but the experimental design would then have made the study much larger due to an increased number of dietary treatments. Nevertheless, based on NSP content, xylanase should be supplemented to wheat DDGS diets at a higher level than used in Chapter 3 to preclude possible limitations in dietary enzyme activity.

In Chapter 3, the potential interaction of xylanase and dietary particle size could have been avoided if the particle size of two major ingredients, wheat and wheat DDGS, was measured to ensure that particles size was equal for both ingredients. Instead, wheat DDGS was included in the diet as it was obtained and particle size was not measured.

In both Chapters, ileal fluid leaked from the cannulae and dropped in to the urine collection trays, contaminating urine as demonstrated by the presence of chromic oxide in several urine samples. Contamination of urine with the liquid portion of digesta probably influenced the data on urinary nutrient excretion.

4.4 Future research

Despite identifying specific characteristics associated with DDGS, the described studies created several new questions that may provide the basis for future research. For the effective utilization of nutrients in DDGS, the nutrient digestibility should be maximized and attention must thus be paid to the factors associated with nutrient digestibility. High NSP content appears to be a major constraint to the nutritional value of DDGS for pigs but the level of supplementary xylanase used in Chapter 3 to reduce the impact of NSP failed to improve nutrient digestibility. Therefore, effects of graded levels of supplementary xylanase on nutrient digestibility of wheat DDGS should be studied. In addition, the content of ferulic acid is negligible in wheat grain, but can be considerable in wheat DDGS due to starch removal. An evaluation of the synergistic effect of ferulic acid esterase and xylanase can thus be worthwhile to determine effects of ferulic acid on xylanase efficacy.

The results of both studies revealed an increase in urinary N excretion with the inclusion of DDGS to the diet, because of increased NPN in DDGS and increased AA catabolism due to the lack of one or more critical AA in DDGS. The low total content and digestibility of the first limiting AA, lysine, in DDGS might mostly be responsible for the AA imbalance. Therefore, a study of beneficial effects of supplementary synthetic AA, such as lysine, to DDGS to correct the AA profile might be interesting.

4.5 Implications and conclusion

The DDGS is a by-product. Results of the present thesis indicate that DDGS can be included in swine rations as an alternative source of energy, protein and P. Accordingly, ingredients such as SBM, canola oil and di-calcium phosphate can partially or entirely be replaced with the addition of DDGS to swine diet. Nevertheless, to be considered as an effective feed ingredient, optimal pig growth and efficiency must be maintained. Based on the total and digestible lysine contents of DDGS and the growth performance of pigs fed DDGS incorporated diets, swine producers need to balance diets properly to ensure that performance is not reduced. While demonstrating the value of DDGS as a potential source of most expensive nutrients in swine nutrition, the findings of this project indicate to ethanol producers the factors that affect the nutritional value of this by-product. The damage of lysine by processing and high NSP content might be the major constraints to the nutritional value of DDGS for pigs. The heat damage of nutrients, lysine in particular, can be minimized and the cost of DDGS production can be reduced with the application of efficient drying technology. The nutritional value of DDGS can be enhanced by reducing the effect of high NSP content. One method to accomplish a higher nutritional value by the ethanol industry level is dehulling the

feedstock grain prior to fermentation thereby eliminating most of the NSP. A second method is application of NSP- degrading enzymes prior to the saccharification process. Such strategies not only improve the nutritional value of DDGS but also maximize the ethanol yield by reducing the effects of NSP on amylase activity during saccharification and by increasing the amount of target substrate in a batch of feedstock used for fermentation.

In conclusion, this research should serve as a good starting point to develop future research aiming at the improvement of the nutritional value of DDGS. As the first step, factors associated with reduced nutrient digestibility and feed intake should be identified for DDGS to play a major role as a potential feed ingredient in Canadian pork industry to reduce feed costs. In addition, the optimum inclusion level of wheat DDGS and xylanase in the diet has yet to be determined and an evaluation of the synergistic effect of xylanase and ferulic acid esterase may be necessary.

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